0031-6997/96/4804-0567$03.00/0
PHARMACOLOGICAL REVIEWS
Vol. 48, No. 4
Printed in U.S.A.

International Union of Pharmacology. XII. Classification of Opioid Receptors[a]

B. N. DHAWAN, F. CESSELIN, R. RAGHUBIR, T. REISINE, P. B. BRADLEY, P. S. PORTOGHESE, AND M. HAMON

Central Drug Research Institute, Chattar Manzil, Lucknow, India (B.N.D., R.R.); Institut National de la Santé et de la Recherche Medicale (INSERM) U288, Faculté de Médecine Pitié-Salpêtrière, Boulevard de l'Hôpital, Paris, France (F.C., M.H.); Department of Pharmacology, University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania (T.R.); Department of Pharmacology, The University of Birmingham, The Medical School, Edgbaston, Birmingham, United Kingdom (P.B.B.); Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota (P.S.P.)

[a] Document prepared by the International Union of Pharmacology (IUPHAR) subcommittee on Opioid Receptors (P. B. Bradley, B. N. Dhawan, C. J. Evans, M. Hamon, H. H. Loh, P. S. Portoghese, R. Raghubir, R. S. Rapaka, T. Reisine and P. W. Schiller).

[b] Address correspondence to: Michel Hamon, Institut National de la Santé et de la Recherche Medicale (INSERM) U288, Faculté de Médecine Pitié-Salpêtrière, 91 Boulevard de l'Hôpital, 75634 Paris cedex 13, France.

I. Introduction

The opioid receptor ligands have their basis in more than 5000 years of medicinal use of opium (from "opos," the Greek word for juice), which is obtained by scoring the unripe seed capsule of poppy *Papaver somniferum* and drying the exudate. The analgesic and anti-diarrheal properties of opium were already recognized by the Sumerians and the early dynastic Egyptians, and the therapeutic use of opium was discussed by Hippocrates, Dioscorides and Galen. Thus, "opium," "laudanum," "pulvis Doveri" and "paregoric" have been used for centuries in western medicine. The nature of the mood changes also produced by opium has been the basis for its non-medicinal use (and abuse). In particular, opium eating and smoking replaced the consumption of alcoholic drinks in Islamic countries, such as Arabia, Turkey and Iran. Opium was also consumed as a favorite substance of pleasure in India and China.

A German chemist, Friedrich Sertüner, isolated the active principle morphine (from "Morpheus," the Greek god of dreams, compound 1 in fig. 1) from opium in 1805, which was then used in therapy. Unfortunately, morphine has just as much potential for abuse as does opium. This prompted medicinal chemists to attempt to develop safer and more efficacious compounds, with the goal of providing analgesia with reduced abuse potential and reduced incidence of side effects (such as respiratory depression). The exercise led to the synthesis of heroin (diacetylmorphine, compound 2 in fig. 1) in 1898, which was claimed to be more potent than morphine and free from abuse liability. This was the first of such claims for

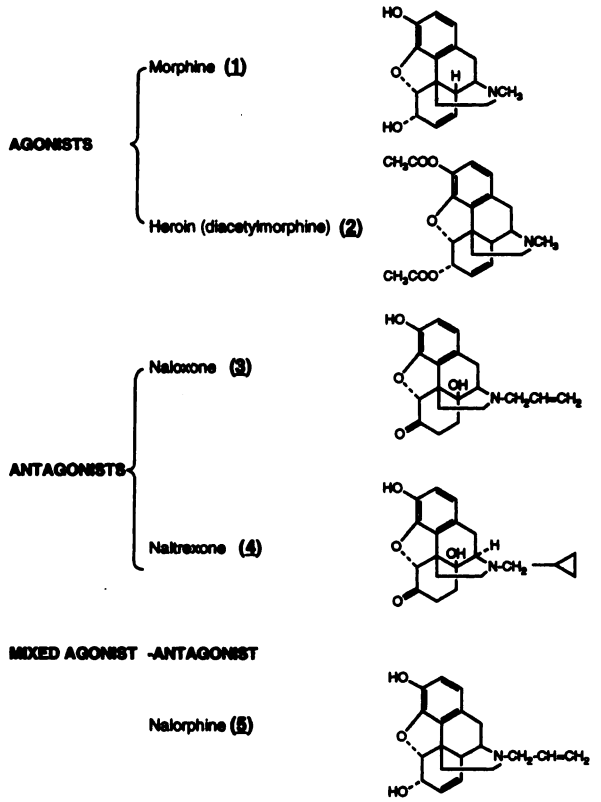

FIG. 1. Non-selective agonists and antagonists at opioid receptors. **Morphine (compound 1)**: (5α,6α)-7,8-didehydro-4,5-epoxy-17-methylmorphinan-3-6-diol. **Heroin (diacetylmorphine) (compound 2)**: (5α,6α)-7,8-didehydro-4,5-epoxy-17-methylmorphinan-3-6-diol diacetate. **Naloxone (compound 3)**: 4,5-epoxy-3,14-dihydroxy-17-(2-propenyl)morphinan-6-one. **Naltrexone (compound 4)**: 17-(cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxymorphinan-6-one. **Nalorphine (compound 5)**: 7,8-didehydro-4,5-epoxy-17-(2-propenyl)morphinan-3,6-diol. Nalorphine is an agonist at OP_2 receptors and an antagonist at OP_3 receptors.

Abbreviations: POMC, proopiomelanocortin; cDNA, complementary deoxyribonucleic acid; IUPHAR, International Union of Pharmacology; DADLE, D-Ala^2-D-leu^5-enkephalin; Tic, tetrahydroisoquinoline; DTLET, Tyr-D-Thr-Gly-Phe-Leu-Thr; DSLET, Tyr-D-Ser-Gly-Phe-Leu-Thr; DSTBULET, Tyr-D-Ser(OtBu)-Gly-Phe-Leu-Thr; BUBU, Tyr-D-Ser(OtBu)-Gly-Phe-Leu-Thr(OtBu); BUBUC, Tyr-D-Cys(StBu)-Gly-Phe-Leu-Thr-(OtBu); DPLPE, Tyr-D-Pen-Gly-Phe-L-Pen; DPDPE, Tyr-D-Pen-Gly-Phe-D-Pen; SNC 80, (±)4-[(α-R)-α-[2S,5R]-4-allyl[2,5-dimethyl-1-piperazinyl]-3-methoxybenzyl]N,N-diethyl-benzamide; SIOM, 7-spiroindanyloxymorphone; NTI, naltrindole; DALCE, [D-Ala^2,Leu^5,Cys^6]enkephalin; BNTX, 7-benzylidenenaltrexone; 5′-NTII, NTI 5′-isothiocyanate; NTB, naltriben; BNTI, N-benzylnaltrindole; i.c.v., intracerebroventricular; IC_{50}, concentration that inhibits to 50%; DAMGO, Tyr-D-Ala-Gly-MePhe-Gly-ol; TENA, 6β,6′β-[ethylenebis (oxyethyleneimino)]bis[17-(cyclopropylmethyl)-4,5α-epoxymorphinan-3,14-diol]; UPHIT, (1S,2S)-trans-2-isothiocyanato-4,5-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide; DIPPA, 2-(3,4-dichlorophenyl)-N-methyl-N-[(1S)-1-(3-isothiocyanatophenyl)-2-(1-pyrrodinyl)ethyl]acetamide; nor-BNI, nor-binaltorphimine; β-FNA, β-funaltrexamine; CTAP, D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2; CTOP, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2; TCTOP, D-Tic-CTOP; [^{125}I]IOXY-AGO, 6β-[^{125}I]-3,14-dihydroxy-17-methyl-4,5α-epoxymorphinan; TRIMU-5, Tyr-D-Ala-Gly-NH-$(CH_2)_2$-$CH(CH_3)_2$; $GABA_A$, γ-aminobutyric acid A; COS, monkey fibroblast cells; CHO, Chinese hamster ovary; TIPP, H-Tyr-Tic-Phe-Phe-OH; TIPPΨ, H-Tyr-TicΨ[CH_2-NH]Phe-Phe-OH; GTP, guanosine triphosphate; cAMP, cyclic adenosine monophosphate; mRNA, messenger ribonucleic acid; ORL_1, Opioid Receptor-Like protein $_1$; OBCAM, Opioid Binding Cell Adhesion Molecule.

novel opioids. However, to date, none has proven valid (see Brownstein, 1993). The first pure opioid antagonist, naloxone (compound 3, and its congener, naltrexone, compound 4 in fig. 1) was produced in the 1940s, after the synthesis of nalorphine (N-allylnormorphine or compound 5 in fig. 1), which was previously used to prevent the effects of opioid receptor agonists. However, this action does not concern all opioid receptors, because nalorphine has also been shown to mimic the action of some agonists (fig. 1).

By the mid-1970s, the first endogenous peptide ligands for opioid receptors (enkephalins and β-endorphin, table 1) were isolated and sequenced (Hughes et al., 1975; Bradbury et al., 1976; Cox et al., 1976; Li and Chung, 1976; Pasternak et al., 1976). Another group of peptides, the first of which was named dynorphin, was then identified in the 1980s (Goldstein et al., 1981, table 1). In the same period, it was recognized that each of the opioid peptides is made as part of a larger precursor protein. In mammals, there are three such precursors: (a) proenkephalin A, which yields four met-enkephalins, one leu-enkephalin, one met-enkephalin-Arg^6-Phe^7 and

TABLE 1
Endogenous ligands of opioid receptors

Mammalian peptides	
Enkephalins	Met^5-enkephalin: Tyr-Gly-Gly-Phe-Met Leu^5-enkephalin: Tyr-Gly-Gly-Phe-Leu Met^5-enkephalin-Arg^6-Phe^7: Tyr-Gly-Gly-Phe-Met-Arg-Phe Met^5-enkephalin-Arg^6-Gly^7-Leu^8: Tyr-Gly-Gly-Phe-Met-Arg-Gly-Leu
Dynorphins	Dynorphin A: Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln Dynorphin B: Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Gln-Phe-Lys-Val-Val-Thr-Arg-Ser-Gln-Glu-Asp-Pro-Asn-Ala-Tyr-Glu-Glu-Leu-Phe-Asp-Val
β-neoendorphin	Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro
β-endorphin (camel)	Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu
Amphibian peptides	
Dermorphins	Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2 Tyr-D-Ala-Phe-Gly-Tyr-Pro-Lys Tyr-D-Ala-Phe-Trp-Tyr-Pro-Asn
Deltorphins	A: Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2 (deltorphin, dermenkephalin, dermorphin gene-associated peptide) B: Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2 (deltorphin II) C: Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2 (deltorphin I)

one met-enkephalin-Arg^6-Gly^7-Leu^8 (Noda et al., 1982); (*b*) prodynorphin (or proenkephalin B), which gives rise to dynorphins A and B, and α- and β-neoendorphins (Kakidani et al., 1982); and (*c*) proopiomelanocortin, which is processed into corticotropin, β-lipotropin and melanotropins along with β-endorphin (Nakanishi et al., 1979, table 1). Among a myriad of potent bioactive substances, the frog skin contains opioid peptides, named dermorphins and deltorphins A, B and C (Erspamer et al., 1989; Lazarus et al., 1994; table 1). All the amphibian opioids have an amino acid with the rare (in a mammalian context) D-enantiomer in lieu of the normal L-isomer. Cloning of the complementary deoxyribonucleic acids (cDNAs) encoding the precursors showed that deltorphin A, on one hand, and deltorphins B and C, on the other hand, derive from different genes (Richter et al., 1990).

Both opiates ("opiate" refers specifically to the products derived from the juice of the opium poppy, although it has been loosely applied to morphine derivatives) and opioids (the term "opioid" refers to any directly acting compound whose effects are stereospecifically antagonized by naloxone), including endogenous opioid peptides, have helped substantially in the identification of opioid receptors. However, the concept of pharmacologically relevant receptors for opioids, based on activities of stereoisomers, was first elaborated by Beckett and Casy as early as 1954. Later, Portoghese (1965) suggested the concept of different modes of interaction of morphine and other analgesics with opioid receptors. Goldstein et al. (1971) subsequently proposed that radiolabeled compounds might be used to demonstrate the existence of these receptors and to characterize them. As soon as radioligands with high specific activities were available, three different groups, independently, but simultaneously, showed that there are stereospecific opioid binding sites in mammalian brain (Pert and Snyder, 1973; Simon et al., 1973; Terenius, 1973).

Although it was becoming clear by the mid-1960s that the actions of opioid agonists, antagonists and mixed agonist-antagonists could be explained best by actions on multiple opioid receptors (Portoghese, 1965), the first convincing evidence for this concept was provided by Martin and coworkers in 1976. Their behavioral and neurophysiological observations in the chronic spinal dog led these authors to propose the existence of three types of opioid receptors. These receptors were named after the drugs used in the studies: mu (μ, for morphine, which induces analgesia, miosis, bradycardia, hypothermia, indifference to environmental stimuli), kappa (κ, for ketocyclazocine, which induces miosis, general sedation, depression of flexor reflexes) and sigma (σ, for SKF 10,047 or N-allylnormetazocine, which induces mydriasis, increased respiration, tachycardia, delirium). After they discovered the enkephalins, Kosterlitz and coworkers (Hughes et al., 1975) studied their properties and those of other opioids using radioligand binding methods and two bioassays with peripheral tissues. Indeed, opioid receptors are present not only in the central nervous system but also at the periphery, and this has been exploited to provide functional models of opioid action. Thus, various preparations of the isolated ileum of the guinea pig and of the vas deferens from mouse, rat, rabbit and hamster have been used for more than 30 years in pharmacological assays to assess the agonist/antagonist properties of opioids. (Kosterlitz et al., 1980, 1981; Wild et al., 1993a). Whereas morphine is more potent than the enkephalins in inhibiting neurotrans-

mitter release giving rise to electrically induced contractions of the guinea pig ileum, the reverse is true in the mouse vas deferens preparation. Moreover, the effects of the opioid peptides on the vas deferens are relatively insensitive to naloxone. Based on these observations, Kosterlitz and coworkers proposed that a fourth type of opioid receptor, named delta (δ, for deferens), is present in mouse vas deferens (Lord et al., 1977). Because the σ receptor has subsequently been shown to be non-opioid in nature (Mannalack et al., 1986), there are thus three main types of pharmacologically defined opioid receptors: μ, δ and κ. Their existence has recently been clearly confirmed using molecular biology approaches. Indeed, three types of opioid receptors have been cloned, with binding and functional properties consistent with their identities as μ-, δ- and κ-opioid receptors, respectively (see Reisine and Bell, 1993; Kieffer, 1995; Satoh and Minami, 1995).

There is some evidence to suggest that additional opioid receptor types may exist. In particular, the epsilon receptor (ϵ, Wuster et al., 1979), the zeta receptor (ζ, Zagon et al., 1991) and a high affinity binding site, lambda (λ, Grevel et al., 1985), may also be parts of the opioid receptor system. Among these putative opioid receptors, the ϵ-receptor has been studied in greater detail, notably in the rat vas deferens. In this organ, β-endorphin is a potent inhibitor of electrically evoked twitching, but shorter sequences than the first 21 amino acids of this peptide are considerably weaker for exerting this effect, and fragments consisting of fewer than 17 amino acids are practically inactive (Schulz et al., 1981). The pharmacological properties of the ϵ-receptor are thus markedly different from those of the other opioid receptors (Schulz et al., 1981; Shook et al., 1988), although some authors have suggested that it might correspond to a subtype of the μ- or the κ-opioid receptors (Nock et al., 1993; Fowler and Fraser, 1994).

With regard to the nomenclature of the well defined opioid receptors, the situation is rather confused for the following reasons: (*a*) although the use of Greek letters is generally accepted by pharmacologists, molecular biologists renamed the δ, κ and μ receptors *DOR*, *KOR* and *MOR*, respectively (table 2); (*b*) both of these nomenclatures are poorly informative regarding the nature of these receptors. Indeed, the Greek letters that derived, for two of them (κ and μ), from synthetic ligands (ketocyclazocine and morphine, respectively), provide no information on the endogenous agonists acting at these receptors. Similarly, the nomenclature proposed by the molecular biologists is not satisfactory because it derives directly from the Greek letters. Based on the guidelines defined by the International Union of Pharmacology (IUPHAR) Committee on Receptor Nomenclature and Drug Classification, receptors should be named after their endogenous ligands and identified by a numerical subscript corresponding to the chronological order of the formal demonstration of their existence by cloning and sequencing (Vanhoutte et al., 1996). Thus, the generic designation for these receptors on which all opioids act as agonists should be OP. Because the δ receptor was the first to be cloned (Evans et al., 1992; Kieffer et al., 1992), it should be renamed OP_1, and the κ and μ receptors, which were then successively cloned (see Reisine and Bell, 1993; Kieffer, 1995; Satoh and Minami, 1995), should become the OP_2 and OP_3 receptors, respectively (table 2). In contrast to the other two nomenclatures used in the literature to date, this new one would allow any newly discovered opioid receptor(s) to be logically named following the same informative guidelines (OP_4, OP_5, etc). IUPHAR guidelines should also be followed for the nomenclature of opioid receptor subtypes, as an additional subscript letter would allow their distinction (OP_{1A} and OP_{1B}, for instance). However, the existence of such subtypes is still largely hypothetical.

This rational nomenclature has been adopted in the subsequent sections devoted to the three opioid receptors whose existence has been firmly established to date.

TABLE 2
Rational (IUPHAR recommendation) and current nomenclatures of opioid receptors

Preferential Endogenous Opioid Ligands	Opioid Receptors		
	IUPHAR recommendation	Pharmacology nomenclature	Molecular biology nomenclature
Enkephalins	OP_1	δ	DOR
Dynorphins	OP_2	κ	KOR
β-endorphin	OP_3	μ	MOR

Current nomenclatures derive from the peripheral preparation that was extensively used for characterizing the receptor (δ, DOR, for mouse vas deferens) or the synthetic ligand that allowed originally its identification (κ, KOR, for ketocyclazocine; μ, MOR, for morphine).

The IUPHAR nomenclature indicates the nature of the endogenous ligand: OP for opioids, and the chronological order of the first formal demonstration of the existence of the receptors. Accordingly, newly identified opioid receptors, if any, would be named OP_4, OP_5, etc.

II. Characterization and Distribution of Opioid Receptors

A. OP_1 (δ) Receptors

1. Agonists at OP_1 receptors. Because the OP_1 receptor was initially defined using the mouse vas deferens preparation, in which enkephalins are more potent than morphine in inhibiting electrically evoked neurotransmitter release (Lord et al., 1977), it is not surprising that these peptides have relatively high affinity (but rather low selectivity) for this receptor (table 2). With few exceptions, all OP_1 receptor agonists are peptides, derived from enkephalins or belonging to the class of amphibian skin opioids (table 1). D-Ala^2-D-leu^5-enkephalin (DADLE) was initially found to be a selective agonist at OP_1 receptor using guinea pig ileum and mouse vas deferens assays (Kosterlitz et al., 1980). However, recep-

tor binding studies subsequently showed that DADLE has only two-fold greater affinity for OP_1 than for OP_3 receptors (James and Goldstein, 1984). A hexapeptide, DSLET (fig. 2), was found to have at least 20-600-fold selectivity, depending on the assay, for OP_1 over OP_2 and OP_3 receptors (Gacel et al., 1980). A related com-

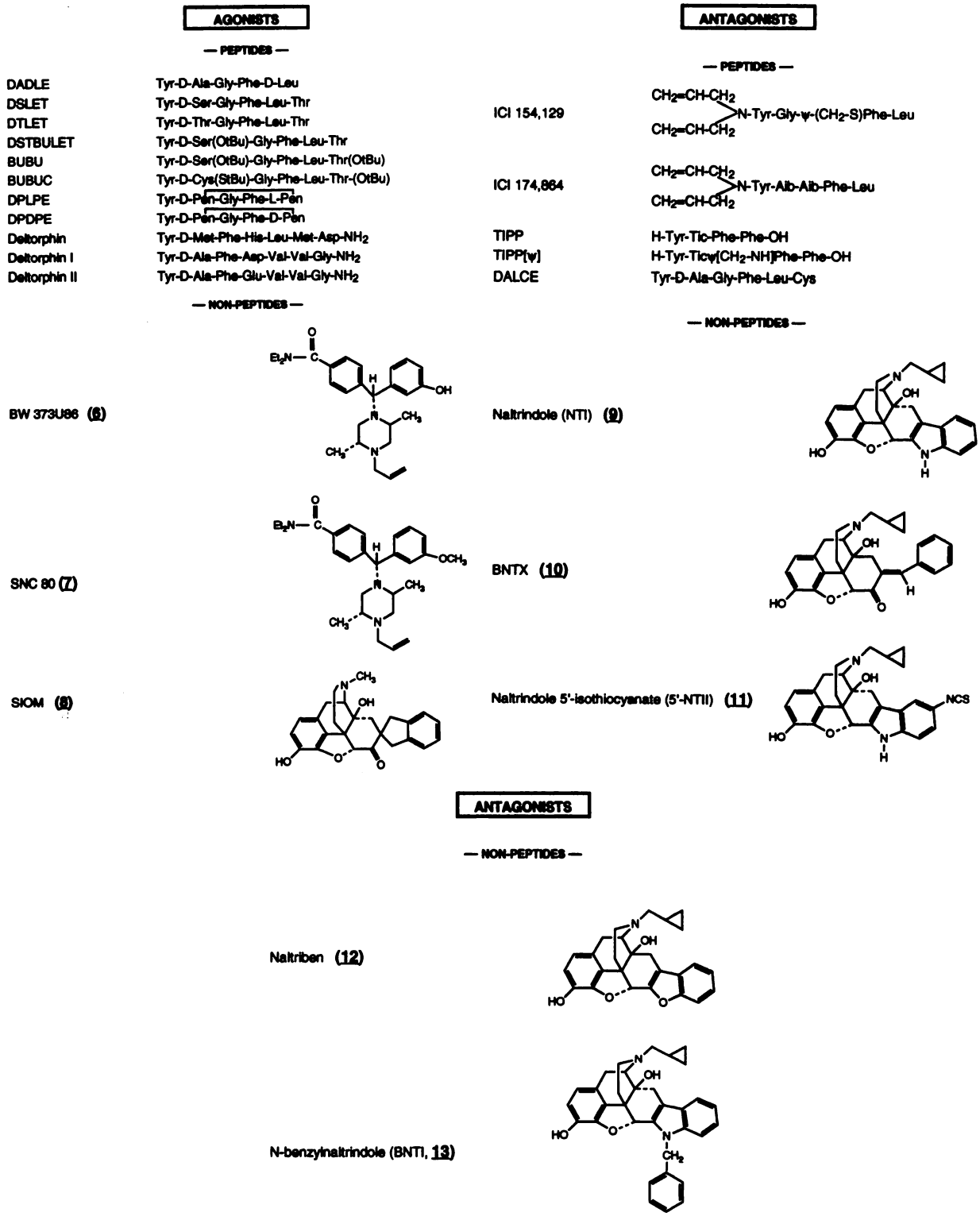

FIG. 2. OP_1 (δ) opioid receptor ligands. **BW373U86 (compound 6)**: (±)4-[(α-R)-α-[2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl]-3-hydroxybenzyl]-N,N-diethyl-benzamide. **SNC 80 (compound 7)**: (±)-4-[(α-R)-α-[2S,5R]-4-allyl-2,5-dimethyl-1-piperazinyl]-3-methoxybenzyl]-N,N-diethyl-benzamide. **SIOM (compound 8)**: 7-spiroindanyloxymorphone. **Naltrindole (NTI) (compound 9)**: 17-cyclo-propylmethyl-6,7-dehydro-4,5-epoxy-3,14-dihydroxy-6,7,2′,3′-indolmorphinan. **BNTX (compound 10)**: 7-benzylidenenaltrexone. **Naltriben (NTB) (compound 12)**: naltrindole benzofuran. Aib, aminoisobutyric acid; Tic, tetrahydroisoquinoline.

pound, DTLET (fig. 2), has three-fold better OP_1 receptor selectivity than does DSLET, as determined in guinea pig ileum and mouse vas deferens assays (Zajac et al., 1983) as well as in receptor binding assays (Delay-Goyet et al., 1985). More recently, novel opioid peptide agonists have been synthesized: DSTBULET (fig. 2), and its analogues BUBU (fig. 2) (Delay-Goyet et al., 1988) and BUBUC (fig. 2) (Gacel et al., 1990), which are up to 1000-fold more potent at OP_1 than at OP_3 receptors. The cyclic peptides, DPLPE (fig. 2), DPDPE (fig. 2) and derivatives, resulting from para-halogen substitution of the Phe ring, are of comparable selectivity for the OP_1 receptors (Mosberg et al., 1983; Toth et al., 1990). Certain opioid peptides from the amphibian skin also have high affinity and selectivity for OP_1 receptors. One of them was named deltorphin by one group (Kreil et al., 1989), but has also been referred to as dermorphin gene-associated peptide (Lazarus et al., 1989), dermenkephalin (Amiche et al., 1989) and more recently, deltorphin A (see Lazarus et al., 1994). Deltorphin I (also referred to as deltorphin C) and deltorphin II (or deltorphin B) (Erspamer et al., 1989; Lazarus et al., 1994), two other amphibian skin peptides, are not only 10-20 fold more selective but also show an affinity for OP_1 receptors that is 10-200 times higher than that of synthetic enkephalin analogues (Erspamer et al., 1989).

The first non-peptidic agonist that was reported to have some selectivity for OP_1 opioid receptors was BW373U86 (fig. 2, compound 6), an opioid with a benzhydrylpiperazine skeleton (Chang et al., 1993). Although membrane binding studies showed that this compound is only approximately 20-fold more potent at OP_1 than at OP_2 and OP_3 receptors (Chang et al., 1993) and exhibits a significant degree of toxicity (Comer et al., 1993), BW373U86 could represent a lead compound for the development of non-peptidic OP_1 receptor agonists. Indeed, the methyl ether of one enantiomer of BW373U86, SNC 80 (fig. 2, compound 7, Bilsky et al., 1995), recently has been synthesized, and its properties are promising. Studies with SNC 80 in the mouse vas deferens and guinea pig ileum preparations as well as radioligand binding assays demonstrated that this compound shows approximately 2000-fold selectivity for OP_1 (rather than OP_3) receptors. Furthermore, in contrast to BW373U86, SNC 80 exhibits only minimal signs of toxicity (Bilsky et al., 1995). As illustrated in figure 2, the naltrexone derivative SIOM is also a potent and rather selective agonist at OP_1 receptors (Portoghese et al., 1993).

2. Antagonists at OP_1 receptors (fig. 2). The first antagonists that were shown to exhibit significant selectivity for the OP_1 receptors were enkephalin analogues. ICI 154,129 (fig. 2) exhibits a 30-fold selectivity for this type of opioid receptors versus the others but is an antagonist of rather low potency (Shaw et al., 1982). ICI 174,864 (fig. 2) has a greater potency at OP_1 receptors (Cotton et al., 1984), but its carboxypeptidase degradation product is an agonist at OP_3 receptors, which makes its use, especially for in vivo investigations aimed at specifically inactivating OP_1 receptors, rather problematic (Cohen et al., 1986). The naltrexone derivative naltrindole (NTI) (fig. 2, compound 9) was the first really selective and potent OP_1 receptor antagonist to be synthesized (Portoghese et al., 1988). It binds to OP_1 receptors in monkey brain membranes with a K_i value in the picomolar range and a 100-fold selectivity for these receptors as compared with OP_2 and OP_3 receptors (Emmerson et al., 1994). In mice, NTI also acts as an agonist at OP_2 receptors, but at higher doses than those for the blockade of OP_1 receptors (Stapelfeld et al., 1992; Takemori et al., 1992). Substitution of tetrahydroisoquinoline (Tic) at the 2-position of a deltorphin-related tetrapeptide analogue produced a potent and highly selective OP_1 receptor antagonist, TIPP (fig. 2) (Schiller et al., 1992). TIPP displaces [^{3}H]-DSLET specifically bound to rat brain membranes with a K_i of approximately 1 nM. It is therefore less potent than NTI, but its selectivity for OP_1 receptors is 80-fold higher than that of the non-peptide antagonist (Schiller et al., 1992). Reduction of the Tic^2-Phe^3 peptide bond in TIPP resulted in TIPP[Ψ] (fig. 2), which shows improved OP_1 antagonist potency and selectivity, and no OP_2 or OP_3 antagonist properties (Schiller et al., 1993). The chemical structures of these various selected OP_1 receptor ligands are given in figure 2.

3. Radioligands and binding assays of OP_1 receptors. The most selective agonist radioligands for the specific labeling of OP_1 receptors are tritiated or radioiodinated derivatives of DPDPE and deltorphins I and II. The affinity of [^{3}H]deltorphins I and II and [^{3}H][4'Cl-Phe^4]DPDPE for OP_1 receptors is one to two orders of magnitude greater than that of [^{3}H]DPDPE (Akiyama et al., 1985; Erspamer et al., 1989; Vaughn et al., 1989; Buzas et al., 1992). ^{125}I-labeled derivatives of deltorphins and DPDPE, which exhibit affinity and selectivity for OP_1 receptors as high as those of the parent compounds (Dupin et al., 1991; Knapp et al., 1991; Fang et al., 1992), have the advantage over tritiated compounds of having, obviously, higher specific radioactivity. Other radioligands of interest for the labeling of OP_1 receptors are the commercially available [^{3}H]DSLET and [^{3}H]DTLET, which are less selective than deltorphin and DPDPE derivatives, but which have high affinities for this receptor and lead to high ratio of specific over total binding (Delay-Goyet et al., 1990). The first antagonist radioligand that was developed for the labeling of OP_1 receptors was [^{3}H]NTI (Yamamura et al., 1992; Contreras et al., 1993). Other potent and selective antagonist radioligands of OP_1 receptors are [^{3}H]TIPP and [^{3}H]TIPP[Ψ] (Nevin et al., 1993, 1995).

a. THE QUESTION OF OP_1 RECEPTOR SUBTYPES. The existence of subtypes of OP_1 receptors was first suggested by Sofuoglu et al. (1991) and Jiang et al. (1991) on the basis of differential blockade of the action of OP_1 receptor

agonists by different OP_1 selective antagonists. Subsequently, studies with brain membranes and with NG 108-15 cells yielded biphasic inhibition of specific OP_1 radioligand binding by various ligands, which led to the proposal of the existence of the so-called "δ_1" and "δ_2" recognition sites (Fang et al., 1994; Fowler and Fraser, 1994). DPDPE would act as a preferential "δ_1" agonist (but also as a partial "δ_2" agonist, Vanderah et al., 1994), whereas DSLET and [D-Ala^2] deltorphin II would be preferential "δ_2" agonists (Portoghese et al., 1992a, b). An oxymorphone derivative, SIOM (fig. 2, compound 8), was recently reported to be the first non-peptide "δ_1" agonist. However, this compound, which acts neither at "δ_2" binding sites nor at OP_2 receptors, is also an OP_3 receptor antagonist (Portoghese et al., 1993). Experiments with antagonists led to the distinction of [D-Ala^2,Leu^5,Cys^6]enkephalin (DALCE) (fig. 2), an irreversible OP_1 receptor antagonist (Bowen et al., 1987), 7-benzylidenenaltrexone (BNTX) (fig. 2, compound 10, Portoghese et al., 1992a), and 7′-substituted glycinate and aspartate conjugates of NTI (Portoghese et al., 1995) as rather selective blockers of the "δ_1" binding site. By contrast, NTI 5′-isothiocyanate (fig. 2, compound 11, 5′-NTII), a non-equilibrium OP_1 receptor antagonist (Portoghese et al., 1992b), naltriben (NTB) (fig. 2, compound 12), a benzofuran analogue of NTI (Sofuoglu et al., 1991), and N-benzylnaltrindole (BNTI) (fig. 2, compound 13, Korlipara et al., 1994), also a long-acting antagonist, would act preferentially at the "δ_2" subtype. According to Tseng et al. (1995), the latter subtype would mediate the effects of met-enkephalin in the spinal cord. However, the actual demonstration of the existence of "δ_1" and "δ_2" subtypes (which should be called OP_{1A} and OP_{1B}, respectively, according to the IUPHAR guidelines, Vanhoutte et al., 1996) requires further investigation, as only one protein with the typical OP_1 receptor pharmacological profile has been cloned to date.

4. Distribution of OP_1 receptors. Similar distribution patterns of OP_1 receptors have been obtained using autoradiographical techniques with various tritiated and radioiodinated ligands (Waksman et al., 1986; Tempel and Zukin, 1987; Mansour et al., 1988; Delay-Goyet et al., 1990; Dupin et al., 1991; Renda et al., 1993). In the central nervous system, OP_1 receptors have a more restricted distribution than other opioid receptors. The highest OP_1 receptor densities are present in olfactory bulb, neocortex, caudate putamen and nucleus accumbens. Thalamus, hypothalamus and brainstem have moderate to poor OP_1 receptor density (Mansour et al., 1988; Dupin et al., 1991; Renda et al., 1993). Recently, antibodies generated against selective portions of the OP_1 receptor amino acid sequence were used to localize this receptor type in the central nervous system of rodents and primates. The observed immunohistochemical distribution matched perfectly that established using autoradiographical methods (Dado et al., 1993; Arvidsson et al., 1995; Bausch et al., 1995; Honda and Arvidsson, 1995). In addition, immunocytochemistry at the ultrastructural level (Cheng et al., 1995) provided the definitive proof of the existence of presynaptic OP_1 receptors responsible for the inhibitory influence of opioids on the release of neurotransmitters (substance P, calcitonin gene-related peptide, etc.) from the terminals of primary afferent fibers within the dorsal horn of the rat spinal cord (Bourgoin et al., 1994).

5. Functions of OP_1 receptors. The OP_1 receptors have a role in analgesia, motor integration, gastro-intestinal motility, olfaction, respiration, cognitive function, mood driven behavior, etc. In rats, selective OP_1 agonists and endogenous enkephalins, through the stimulation of OP_1 receptors, have been shown to increase locomotor activity and to induce antidepressant-like effects (which are dependent on dopaminergic systems; Baamonde et al., 1992). In addition, OP_1 receptors are expressed by immune cells in line with data showing that endogenous opioids acting at these receptors can affect immune functions (Hamon, 1991). Spinal OP_1 receptors are involved in the antinociceptive action of opioids (Porreca et al., 1984, 1987; Sullivan et al., 1989; Drower et al., 1991; Improta and Broccardo, 1992; Stewart and Hammond, 1993), notably through the mediation of a direct inhibitory action of selective agonists on the release of substance P and calcitonin gene-related peptide from the terminals of nociceptive primary afferent fibers (Bourgoin et al., 1994). When administered onto the spinal cord, OP_1 receptor agonists appear particularly effective toward thermal and chemical stimuli (Schmauss and Yaksh, 1984; Porreca et al., 1987; Paul et al., 1989). The spinal sites of action of OP_1 receptor agonists for reducing nociception do not exclude the involvement of supraspinal and peripheral (see Stein, 1993) OP_1 receptors in their analgesic effects. Indeed, Mathiasen and Vaught (1987), Heyman et al. (1988) and Jiang et al. (1990) provided evidence for the involvement of supraspinal receptors in analgesia due to OP_1 receptor agonists. Furthermore, in mice that are deficient in OP_3 receptors, intracerebroventricular (i.c.v.) administration of morphine or DAMGO is ineffective in producing antinociception, while the potency of OP_1 receptor agonists such as DPDPE is unaltered (Vaught et al., 1988). OP_1 receptor stimulation also produces respiratory depression (Haddad et al., 1984; Morin-Surun et al., 1984; Pazos and Florez, 1984; Yeadon and Kitchen, 1990; Freye et al., 1991). Treatment with OP_1 agonists can lead to a reduced respiratory frequency with a prolongation of expiratory time (Haddad et al., 1984; Morin-Surun et al., 1984). Both peripheral (Fox-Threlkeld et al., 1994; Pol et al., 1994) and central (spinal and supraspinal) (Burks et al., 1988; Broccardo and Improta, 1992; Pol et al., 1994) OP_1 receptors seem to be involved in the inhibition of gastrointestinal transit by selective agonists. Medullary OP_1 receptors are also important for cardiovascular regulation (Srimal et al., 1982; Arndt, 1987)

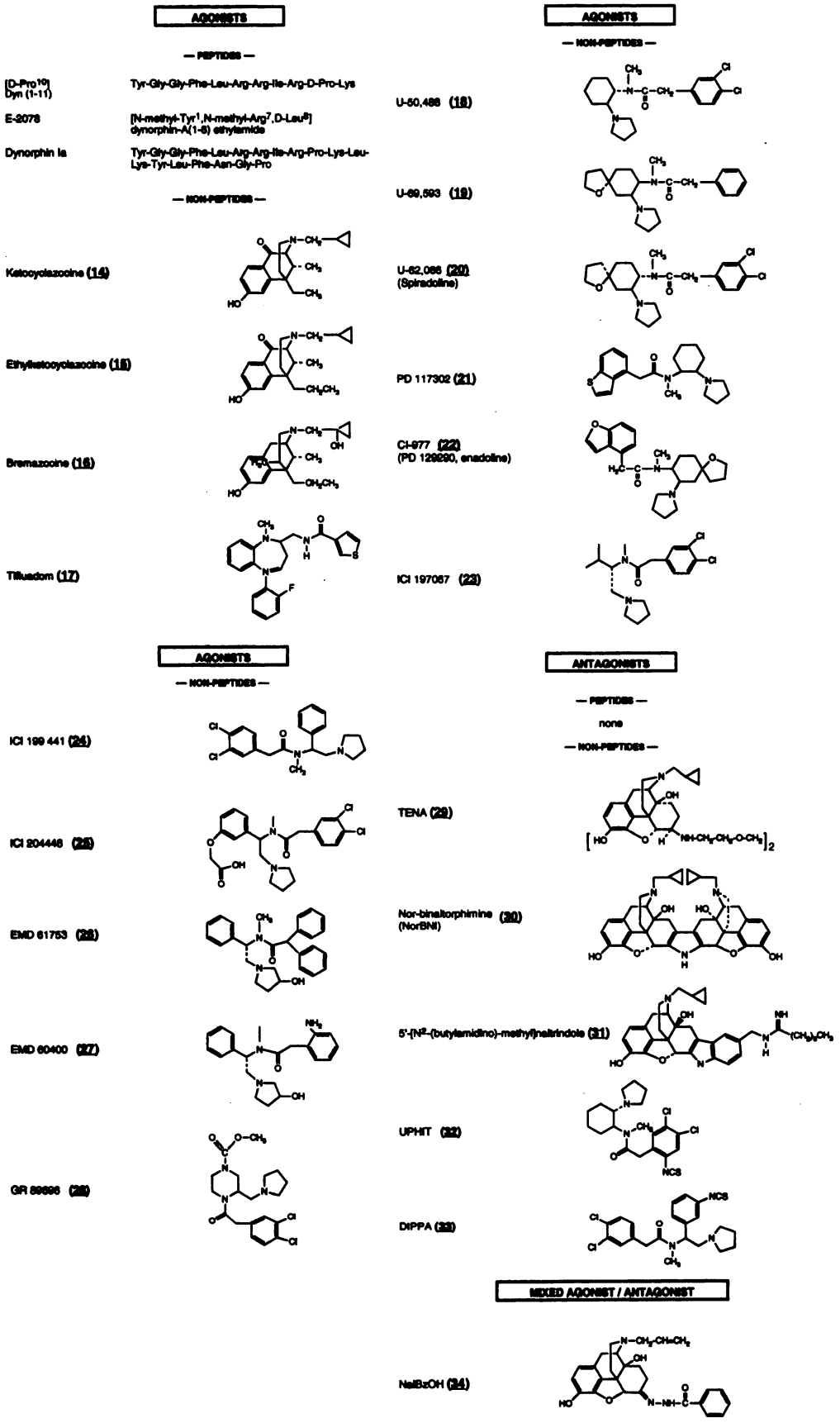

FIG. 3. OP_2 (κ) opioid receptor ligands. **Ketocyclazocine (compound 14)**: 3-(cyclopropylmethyl)-8-keto-1,2,3,4,5,6-hexahydro-6,11-dimethyl-2,6-methano-3-benzazocin-8-ol. **Ethylketocyclazocine (compound 15)**: 3-(cyclopropylmethyl)-8-keto-1,2,3,4,5,6-hexahydro-6-methyl-11-ethyl-2,6-methano-3-benzazocin-8-ol. **Bremazocine (compound 16)**: (±)-6-ethyl-1,2,3,4,5,6-hexahydro-3-[(1-hydroxycyclopropyl)-

and appear to participate in the central hypotensive effect of clonidine (Raghubir et al., 1987).

B. OP_2 (κ) Receptors

1. Agonists at OP_2 receptors. The OP_2 receptor was originally defined by the unique in vivo pattern of agonist activity of ketocyclazocine (fig. 3, compound 14), which differs markedly from that of morphine. Thus, in the seminal work of Martin et al. (1976), flexor reflex depression and sedation without marked effects on heart rate or the skin twitch reflex were specifically ascribed to the activation of OP_2 receptors by ketocyclazocine. This benzomorphan, together with ethylketocyclazocine (fig. 3, compound 15, Martin et al., 1976; Lord et al., 1977) and other compounds initially used for studying OP_2 receptors, such as bremazocine (fig. 3, compound 16, Römer et al., 1980) and tifluadom (fig. 3, compound 17, a benzodiazepine derivative, Römer et al., 1982) have generally high affinity for opioid receptors but are rather non-selective OP_2 agonists (Emmerson et al., 1994). The first really selective (OP_2:OP_3 selectivity ratio of 50-200) OP_2 agonist, the arylacetamide U-50,488 (fig. 3, compound 18), was synthesized in 1982 by the Upjohn Company (Kalamazoo, MI) (Lahti et al., 1982; Von Voigtlander et al., 1983). It was followed by compounds U-69,593 (fig. 3, compound 19, Lahti et al., 1985) and U-62,066 (fig. 3, compound 20, spiradoline, Von Voigtlander and Lewis, 1988) with comparable (Emmerson et al., 1994; France et al., 1994) or higher OP_2 selectivity (Lahti et al., 1985).

Several other agonists have been synthesized as derivatives of this first series of arylacetamide compounds. These include PD 117302 (fig. 3 compound 21, Clark et al., 1988), CI-977 (fig. 3 compound 22, or PD 129290 or enadoline, Hunter et al., 1990), ICI 197067 (fig. 3, compound 23), ICI 199441 (fig. 3, compound 24), and ICI 204448 (fig. 3 compound 25, Costello et al., 1988; Nock et al., 1989). Contrary to ICI 197067 (fig. 3, compound 23) which readily crosses the blood-brain barrier, ICI 204448 (fig. 3, compound 25) does not substantially enter the brain (Barber et al., 1994a, b). EMD 61753 (fig. 3, compound 26), and, to a lesser extent, EMD 60400 (fig. 3, compound 27) are also selective OP_2 receptor agonists acting exclusively at the periphery (Barber et al., 1994a, b). A series of benzeneacetamido-piperazine analogues, such as GR 89696 (fig. 3, compound 28), are also potent and rather selective OP_2 receptor agonists (Hayes et al., 1990; Rogers et al., 1992).

The most probable endogenous ligands of OP_2 receptors are dynorphins (table 2, Chavkin et al., 1982). Dynorphins A and B (table 1) have high affinity (K_i = 1.1 nM), but limited selectivity, for OP_2 receptors (Corbett et al., 1982). Various structural modifications have been made in dynorphin molecules in attempts to synthesize analogues with enhanced selectivity for the OP_2 receptors. Thus, [D-Pro^{10}]dynorphin A-(1-11) (fig. 3) was shown to be about 200-fold more potent than U-50,488 (fig. 3, compound 18) in stimulating OP_2 receptors (Gairin et al., 1985). More recently synthesized dynorphin A-(1-11) derivatives are undoubtedly selective OP_2 agonists (OP_2:OP_3:OP_1 K_i ratio = 1/1000/7000), but their biological activities are still poorly characterized (Choi et al., 1992; Lung et al., 1995). Shorter (E-2078, fig. 3, Yoshino et al., 1990) and longer (dynorphin Ia, fig. 3, Martinka et al., 1991) dynorphin A analogues with potent and selective OP_2 agonist properties have also been described.

2. Antagonists at OP_2 receptors (fig. 3). The first compounds designed for blocking OP_2 receptors, such as TENA (fig. 3, compound 29), lacked sufficient selectivity (Kosterlitz et al., 1981; Portoghese and Takemori, 1985). However, the concept of bivalent ligands, used for the synthesis of TENA, led to the morphine derivative norbinaltorphimine (nor-BNI) (fig. 3, compound 30, Portoghese et al., 1987), which has a K_i value for inhibiting [^{3}H]U-69,593 binding to monkey brain membranes of 60 pM, and a 100-fold and 200-fold preference for OP_2 over OP_1 and OP_3 receptors, respectively (Emmerson et al., 1994). In vivo, nor-BNI exhibits a unusually long duration of action as OP_2 receptor antagonist (Horan et al., 1992), and its OP_2 selectivity has been questioned (Birch et al., 1987; Levine et al., 1990; Spanagel et al., 1994).

methyl)]-11,11-dimethyl-2,6-methano-3-benzazocin-8-ol. **Tifluadom (compound 17)**: (±)-N-[(5-(O-fluorophenyl)-2,3-dihydro-1-methyl-1H-1,4-benzodiazepin-2yl)methyl]-3-thiophenecarboxamide. **U-50,488 (compound 18)**: trans-3,4-dichloro-N-methyl-N[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide. **U-69,593 (compound 19)**: (5α,7α,8β)-(−)-N-methyl-N[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl]-phenyl-benzene acetamide. **U-62,066 (spiradoline) (compound 20)**: (5α,7α,8β)-(±)-3,4-dichloro-N-[7-(1-pyrrolidinyl)-1-oxaspirol(',5)dec-8-yl]methan sulfonate. **PD 117,302 (compound 21)**: (±)-trans-N-methyl-N-[2-1-pyrrolidinyl)-cyclohexyl]benzo[b]thiophene-4-acetamide. **CI-977 (PD 129,290, enadoline) (compound 22)**: (5R)-(5α,7α,8β)-N-methyl-N[7-(1-pyrrolidinyl)-1-oxaspirol [4,5]dec-8-yl]-4-benzofuranacetamide. **ICI 197,067 (compound 23)**: (2S)-N-[2-(N-methyl-3,4-dichlorophenylacetamido)-3-methylbutyl]-pyrrolidine. **ICI 199,441 (compound 24)**: 2-(3,4-dichlorophenyl)-N-methyl-N-[(1S)-1-phenyl-2-(1-pyrrolidinyl)ethyl]acetamide. **ICI 204,448 (compound 25)**: 2-[3-(1-(3,4-dichlorophenyl-N-methylacetamido)-2-pyrrolidinoethyl)-phenoxy] acetic acid. **EMD 61,753 (compound 26)**: N-methyl-N-[(1S)-1-phenyl-2((3S)-3-hydroxypyrrolidine-1-yl)-ethyl]-2,2-diphenylacetamide. **EMD 60,400 (compound 27)**: N-methyl-N-[(1S)-1-phenyl-2-((3S)-hydroxypyrrolidine-1-yl)-ethyl]-2-amino-phenylacetamide. **GR 89,696 (compound 28)**: methyl-4-[(3,4-dichlorophenyl)acetyl]-3-(1-pyrrolidinylmethyl)-1-piperazinecarboxylate. **TENA (compound 29)**: 6β,6'β-[ethylenebis (oxyethyleneimino)]bis[17-(cyclopropylmethyl)-4,5α-epoxymorphinan-3,14-diol]. **Nor-binaltorphimine (nor-BNI) (compound 30)**: 17,17'-bis(cyclo-propylmethyl)-6,6',7,7'-tetrahydro-4,5,4',5'-diepoxy-6,6'-(imino)[7,7'-bimorphinan]-3,3',14,14'-tetrol. **UPHIT (compound 32)**: (1S,2S)-trans-2-isothiocyanato-4,5-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide. **DIPPA (compound 33)**: 2-(3,4-dichlorophenyl)-N-methyl-N-[(1S)-1-(3-isothiocyanatophenyl)-2-(1-pyrrodinyl)ethyl]acetamide. **NalBzOH (compound 34)**: 6-desoxy-6-benzoyl-hydrazido-N-allyl-14-hydroxy-dihydronormorphinone.

More recently, Olmsted et al. (1993) synthesized a new series of NTI derivatives, among which 5'-[N^2-(butylamidino)-methyl] NTI (fig. 3, compound 31) revealed to be more potent than nor-BNI to block OP_2 receptors. In addition, this compound is rather selective, because it exhibits a 57-fold and 90-fold preference for OP_2 over OP_3 and OP_1 receptors, respectively (Olmsted et al., 1993). Also included in figure 3 are UPHIT (fig. 3, compound 32) and DIPPA (fig. 3, compound 33), two derivatives of U-50488 (fig. 3, compound 18), which were reported to be selective and irreversible antagonists at OP_2 receptors (De Costa et al., 1989; Chang et al., 1994).

3. Radioligands and binding assays of OP_2 receptors. Initial studies describing the distribution and the binding characteristics of the OP_2 receptors have used nonselective opioid radioligands, such as the oripavine [^{3}H]etorphine (Audigier et al., 1982) and the benzomorphans [^{3}H]ethylketocyclazocine (fig. 3 compound 15, Gillan et al., 1980) and [^{3}H]bremazocine (fig. 3 compound 16, Kosterlitz et al., 1981), as well as tritiated dynorphins (Gillan et al., 1985), in the presence of "cold" ligands to saturate the other opioid receptors. Now, tritium-labeled selective ligands such as [^{3}H]PD 117,302 ((fig. 3 compound 21, Clark et al., 1988), [^{3}H]CI-977 (fig. 3 compound 22, Boyle et al., 1990), [^{3}H]U-69,593 (fig. 3 compound 19, Lahti et al., 1985), and [^{3}H]nor-BNI (fig. 3 compound 30, Marki et al., 1995) are available for the specific labeling of OP_2 receptors in brain membranes and sections. [^{3}H]CI-977 (fig. 3 compound 22) is probably the best radioligand available to date, with an affinity for OP_2 receptors in both guinea pig and rat brain homogenates ten-fold higher than that of [^{3}H]U-69,593 (fig. 3 compound 19, K_d = 0.1-0.2 nM for [^{3}H]Cl-977 versus 1-3 nM for [^{3}H]U-69,593, Boyle et al., 1990).

a. THE QUESTION OF OP_2 RECEPTOR SUBTYPES. Binding studies with brain membranes yielded multiphasic inhibition curves suggesting that the selective arylacetamide agonists, U-50,488 (fig. 3 compound 18), U-69,593 (fig. 3 compound 19) and CI-977 (fig. 3, compound 22), bind only to the "κ_1" subtype of OP_2 receptors, whereas benzomorphan ligands also interact with their "κ_2" and "κ_3" subtypes (Clark et al., 1989; Nock et al., 1990; Horan et al., 1991, 1993). UPHIT (fig. 3, compound 32) would block preferentially the "κ_1" sites, whereas nor-BNI (fig. 3, compound 30) would act at both "κ_1" and "κ_2" sites (Horan et al., 1991). NalBzOH (fig. 3, compound 34), a mixed agonist/antagonist benzoylhydrazone derivative of naloxone (Price et al., 1989), would be a rather selective "κ_3 agonist" in mice (Paul et al., 1990), but not in rhesus monkeys (France and Woods, 1992), and a "κ_1 antagonist." However, this compound also acts as an antagonist at OP_1 and OP_3 receptors (Paul et al., 1990).

To date, however, the pharmacological profiles of "κ_2" and "κ_3" binding sites remain poorly defined. Some authors (Nock et al., 1990, 1993; Fowler and Fraser, 1994) proposed that they might in fact correspond to the ϵ- and/or the "μ_2" receptors because of their relatively high affinity for β-endorphin and/or DAMGO. Alternatively, the so-called subtypes of "κ" receptor (and of other opioid receptors) could, more probably, correspond to different affinity states of the same receptor, depending on its coupling with G protein (Richardson et al., 1992). In any case, no cloning data have yet been provided that support the existence of OP_2 receptor subtypes.

4. Distribution of OP_2 receptors. Due to the possible existence of arylacetamide-sensitive and -insensitive OP_2 binding sites, it is not surprising that the distributions of specific binding sites for tritiated arylacetamide derivatives and benzomorphans present some differences (Nock et al., 1988). In addition, species differences are particularly striking (see Zukin et al., 1988; Boyle et al., 1990; Rothman et al., 1992). For instance, in the guinea pig, the highest density of specific sites for the tritiated arylacetamide compounds ("κ_1" sites) is found in the inner layers of the cerebral cortex, the substantia nigra and the interpeduncular nucleus. By contrast, in the rat, only low levels of labeling by these radioligands are found throughout the cerebral cortex, the highest densities of specific binding sites being observed in the nucleus accumbens, claustrum, dorsal endopiriform nucleus and interpeduncular nucleus (Nock et al., 1988; Boyle et al., 1990). Furthermore, in the latter species, no area caudal to the forebrain was heavily labeled (Nock et al., 1988).

5. Functions of OP_2 receptors. OP_2 receptors have been implicated in the regulation of several functions. These include nociception, diuresis, feeding and neuroendocrine secretions (Hansen and Morgan, 1984). In addition, recent evidence of the expression of OP_2 receptors by lymphoma cells (Hom et al., 1995) suggests that these receptors also participate in the control of immune function. OP_2 receptor agonists have antinociceptive properties in rodents and rhesus monkeys (Porreca et al., 1987; Schmauss, 1987: Millan, 1989; Millan et al., 1989; Nakazawa et al., 1991; France et al., 1994). However, contradictory data have been published concerning the nature of the nociceptive stimuli against which OP_2 receptor agonists are particularly effective (Porreca et al., 1987; Schmauss, 1987; Millan, 1989). Whereas a spinal site of action for the analgesic effects of OP_2 agonists seems to be established, the existence of additional supraspinal sites that may be involved in these effects is still controversial (Porreca et al., 1987; Schmauss, 1987; Millan et al., 1989; Nakazawa et al., 1991). Apparently, both central and peripheral OP_2 receptors mediate the anti-diarrheal properties of opioids (Hansen and Morgan, 1984). Increased urination induced by OP_2 agonists appears to be due to an inhibition of the release of antidiuretic hormone from the neurohypophysis upon OP_2 receptor stimulation (Leander, 1983). OP_2 receptors could also be involved in thermoregulation (Handler et al., 1992) and modulation of cardiorespiratory function in the rat (Hassen et al., 1984a). However, among opioid receptor agonists, those acting

selectively at OP_2 receptors have limited effects, on respiratory function, especially in non-human primates (Martin et al., 1976; France et al., 1994). In contrast to OP_3 receptor agonists, OP_2 receptor agonists do not have positive subjective effects in non-human species (Mucha and Herz, 1985) and can produce dysphoria in humans (Pfeiffer et al., 1986).

C. OP_3 (μ) Receptors

1. Agonists at OP_3 receptors. (fig. 4) The present knowledge of the pharmacological properties of the OP_3 receptors has been largely derived from studies with the guinea pig ileum, which is rich in this type of receptors. Their stimulation by opioid receptor agonists inhibits

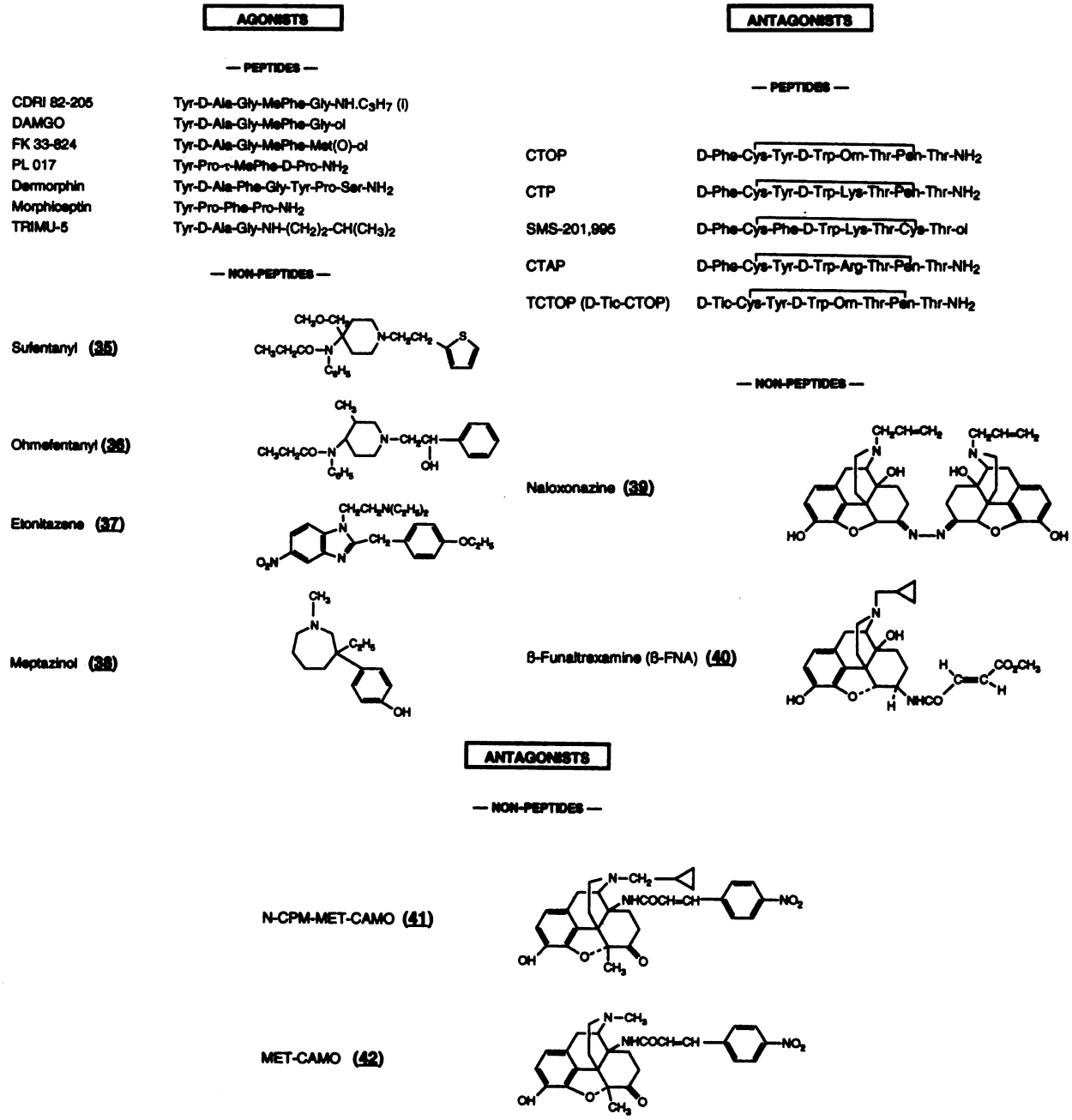

FIG. 4. OP_3 (μ) opioid receptor ligands. **Sufentanyl (compound 35)**: N-[4-(methoxymethyl)-1-[2-(2-thienyl)ethyl]-4-piperidinyl]-N-phenyl-propanamide. **Ohmefentanyl (compound 36)**: N-[1-(β-hydroxy-β-phenethyl)-3-methyl-4-piperidyl]-N-phenylpropionamide. **Etonitazene (compound 37)**: 2[(4-ethoxyphenyl)methyl]-N,N-diethyl-5-nitro-1H-benzimidazole-1-ethan-amine. **Meptazinol (compound 38)**: m-(3-ethyl-1-methyl-hexahydro-1-H-azepin-3-yl)phenol. **Naloxonazine (compound 39)**: bis[5-α-4,5-epoxy-3,14-dihydroxy-17(2-propenyl)-morphinan-6-ylidene]hydrazine. **β-funaltrexamine ([beta-FNA) (compound 40)**: (E)-4[[5α,6β)-17-(cyclo-propylmethyl)-4,5-epoxy-3,14-dihydroxymorphinan-6-yl]amino]-4-oxo-2-butenoic acid methyl ester. **N-PCM-MET-CAMO (compound 41)**: N-cyclopropylmethylnor-5β-methyl-14-(p-nitrocinnamoylamino)-7,8-dihydromorphinone. **MET-CAMO (compound 42)**: 5β-methyl-14β(p-nitrocinnamoylamino)-7,8-dihydromorphinone.

neurotransmitter release (and the resulting muscle contraction) normally triggered by electrical field stimulation. The OP_3 receptor pharmacological profile has generally been characterized in comparison with that of the OP_1 receptor, for which the preferential peripheral tissue preparations are the mouse vas deferens [in which this type of receptors was defined, but where OP_1 and OP_2 receptors are also expressed (Hutchison et al., 1975; Lord et al., 1977)], and the hamster vas deferens, which seems to contain a more "pure" population of OP_1 receptors (McKnight et al., 1985).

The alkaloid morphine (fig. 1, compound 1) has an approximately 50-fold higher affinity for OP_3 than for OP_1 receptors (Emmerson et al., 1994). Among the nonpeptide drugs, the piperidine derivative sufentanyl (fig. 4, compound 35) is a potent opioid agonist with high affinity and selectivity for the OP_3 receptor (Magnan et al., 1982; Emmerson et al., 1994). One of its derivatives, ohmefentanyl (fig. 4, compound 36), was claimed to be the opioid agonist with the highest affinity and selectivity for OP_3 receptors (Xu et al., 1985; Goldstein and Naidu, 1989). However, this compound, as well as various other fentanyl derivatives, appears to bind also to "σ" receptors (Wang et al., 1991). To date, the most potent and selective agonist at OP_3 receptors is the benzimidazole opioid etonitazene (fig. 4, compound 37), with a K_d value of 20 pM for OP_3 binding sites in monkey brain membranes, and OP_3:OP_1 and OP_3:OP_2 selectivities of about 9000 and 12,000, respectively (Emmerson et al., 1994).

FK 33,824 (fig. 4, Roemer et al., 1977) was the first peptide analogue of met-enkephalin with high affinity for the OP_3 receptor, and OP_3:OP_1 selectivity of approximately 30 (McKnight and Rees, 1991), to be synthesized. The related compound DAMGO (fig. 4, also referred to as DAGO or DAGOL, Handa et al., 1981), which has become the most commonly used selective OP_3 receptor agonist, is almost 10 times more selective than FK 33,824 and has high affinity (K_d = 0.7 nM) for the OP_3 receptor (Mansour et al., 1986; Hawkins et al., 1988). These properties led to the development of [^{3}H]DAMGO for the selective labeling of OP_3 receptors in membranes or sections from various tissues. Another enkephalin analogue, CDRI 82-205 (fig. 4), is also a rather selective OP_3 receptor agonist (Raghubir et al., 1988). Synthesized on the basis of morphiceptin, PL017 (or PL17, fig. 4), a tetrapeptide derived from β-casein having selectivity but low affinity for OP_3 receptors (Chang et al., 1981), exhibits improved characteristics with IC_{50} values of 5.5 nM and > 10,000 nM for inhibiting the specific binding to rat brain membranes of [^{125}I]-FK 33,824, as OP_3 receptor radioligand, and [^{125}I]-DADLE, as OP_1 receptor radioligand, respectively (Chang et al., 1983). Finally, dermorphins, the naturally occurring amphibian heptapeptides, and their related carboxyl-terminal amides, have high affinity and selectivity for OP_3 receptors (Richter et al., 1990). They show an affinity for the preferred OP_3 site 2 to 4 orders of magnitude greater than their affinity for the OP_1 and OP_2 sites (Negri et al., 1992).

2. Antagonists at OP_3 receptors (fig. 4). Naloxone (fig. 1, compound 3), the first opioid receptor antagonist identified, has higher affinity for the OP_3 receptor than for the other opioid receptors (Magnan et al., 1982; Emmerson et al., 1994). Thus, a careful dose selection of this drug can allow the complete blockade of OP_3 receptors with only negligible antagonism at OP_1 and OP_2 receptors. Naltrexone (fig. 1, compound 4) is less OP_3 receptor-selective (Magnan et al., 1982; Emmerson et al., 1994) but has a greater potency and longer duration of action than naloxone (see Crabtree, 1984). Other long-lasting OP_3 receptor antagonists are naloxazone and naloxonazine (fig. 4, compound 39), the former perhaps acting by spontaneous rearrangement of the azine (Hahn and Pasternak, 1982), which have been characterized as relatively selective for a putative OP_3 receptor subtype ("μ_1"). The fumarate methyl ester derivative of naltrexone, β-funaltrexamine (β-FNA) (fig. 4, compound 40, Portoghese et al., 1980), acts as an irreversible OP_3 antagonist, but also as a reversible OP_2 agonist (Ward et al., 1985). More recently developed derivatives of naltrexone (N-CPM-MET-CAMO, fig. 4, compound 41) and dihydromorphinone (MET-CAMO, fig. 4, compound 42), containing a cinnamoylamino group, appear to be selective irreversible antagonists at OP_3 receptors without exerting any agonistic action at other opioid receptors (Jiang et al., 1994).

Antagonists with the highest selectivity toward OP_3 receptors are cyclic peptides related to somatostatin (Pelton et al., 1986; Kazmierski et al., 1988). The most frequently used compounds are CTAP and CTOP (fig. 4), which inhibit [^{3}H]naloxone binding to rat brain membranes with an IC_{50} value of about 3 nM and have a 1200- and 4000-fold selectivity for the OP_3 versus the OP_2 and OP_1 receptors (Pelton et al., 1986). The recently designed analogue D-Tic-CTOP (TCTOP) has about 10,000-fold higher affinity for OP_3 than for OP_1 receptors (Kazmierski et al., 1988). The chemical structures of selected OP_3 agonists and antagonists are given in figure 4.

3. Radioligands and binding assays of OP_3 receptors. Tritiated fentanyl derivatives (Leysen et al., 1983; Wang et al., 1991; Fitzgerald and Teitler, 1993), [^{3}H] or ^{125}I-FK 33-824 (Moyse et al., 1986), [^{3}H]PL017 (Blanchard et al., 1987), [^{3}H]β-FNA (Liu-Chen et al., 1991) and especially [^{3}H]DAMGO (Handa et al., 1981) have been used—and are still used—as agonist radioligands for the OP_3 receptor. Now, the ^{3}H derivative of the antagonist CTOP offers better OP_3 receptor selectivity (Hawkins et al., 1989). The usefulness of the very recently synthesized naltrexone derivative, [^{125}I]IOXY-AGO, as a potent and selective radioligand of OP_3 receptors deserves further investigation (Xu et al., 1995).

a. THE QUESTION OF OP_3 RECEPTOR SUBTYPES. On several occasions, binding assays with brain membranes gave biphasic inhibition curves suggesting the existence of two subtypes, called "μ_1" and "μ_2", of the OP_3 receptors. According to Pasternak and his colleagues (see Pasternak and Wood, 1986), the "μ_2" subtype would correspond to the OP_3 receptor, as defined from pharmacological studies with the guinea pig ileum, whereas the "μ_1" subtype would have a different pharmacological profile. In particular, the latter subtype would exhibit a five-fold higher affinity for DAMGO than the "μ_2" subtype. Furthermore, meptazinol (fig. 4 compound 38, Spiegel and Pasternak, 1984) and etonitazene (fig. 4 compound 37, Moolten et al., 1993) would be preferential "μ_1" agonists, and, as already emphasized, naloxazone and naloxonazine (fig. 4, compound 39) would be preferential "μ_1" antagonists (see Pasternak and Wood, 1986). However, Cruciani et al. (1987) could not confirm that naloxonazine (fig. 4, compound 39) binds selectively (and irreversibly) to "μ_1" receptors. The enkephalin analog Tyr-D-Ala-Gly-NH-$(CH_2)_2$-$CH(CH_3)_2$ (TRIMU-5) (fig. 4, Gacel et al., 1988) would be a rather selective "μ_2" agonist (Tive et al., 1992) with antagonist properties at "μ_1" receptors (Pick et al., 1992). However, no support for the existence of OP_3 receptor subtypes has yet been obtained from molecular biology investigations. Indeed, it is probable that these subtypes correspond in fact to the same receptor protein, which is either coupled to G protein or uncoupled in the plasma membrane. Alternatively, they might also correspond to the coupling of the same receptor with different G proteins.

4. Distribution of OP_3 receptors. As shown by autoradiographical studies with selective radioligands, OP_3 receptors are distributed throughout the neuraxis. The highest density of these receptors is present in the caudate putamen, where they exhibit a typical patchy distribution (in the rat). OP_3 receptor density then diminishes in the following order: neocortex, thalamus, nucleus accumbens, hippocampus and amygdala. OP_3 receptors are also present in the superficial layers of the dorsal horn of the spinal cord, where they are located, at least in part, on the presynaptic terminals of nociceptive primary afferent fibers (Besse et al., 1990). Moderate concentrations are found in the periaqueductal gray and raphe nuclei, and low density is seen in the hypothalamus, preoptic area and globus pallidus (Waksman et al., 1986; Hawkins et al., 1988; Mansour et al., 1988). Recently, the distribution, in the rat brain, of immunoreactivity to antibodies generated against a peptide sequence present in a purified "μ"-opioid binding protein was shown to be concordant with the distribution of OP_3 receptors (Hiller et al., 1994). More generally, immunocytochemical investigations with antibodies raised against specific portions of the amino acid sequence of the OP_3 receptor fully confirmed the autoradiographical data. In particular, immunocytochemical labeling was found on the terminals of primary afferent fibers within the dorsal horn of the spinal cord, in agreement with the inference, based on biochemical and electrophysiological observations, of their presynaptic location on the fibers conveying nociceptive signals (Besse et al., 1990; Bourgoin et al., 1994; Honda and Arvidsson, 1995).

As already mentioned, OP_3 receptors are also widely distributed in the peripheral nervous system. In particular, myenteric neurons in the gut (Hutchison et al., 1975), and the vas deferens (Lemaire et al., 1978), in the rat, have been shown to express these receptors.

5. Functions of OP_3 receptors. Highly selective OP_3 receptor agonists are potent antinociceptive drugs, indicating that OP_3 receptors, located in both spinal and supraspinal structures (Chaillet et al., 1984; Porreca et al., 1984, 1987; Fang et al., 1986; Paul et al., 1989), as well as at the periphery (see Stein, 1993), play an important role in the control of nociception (Hansen and Morgan, 1984). OP_3 receptor agonists block the nociceptive responses to mechanical, thermal or chemical high intensity stimulations (Knapp et al., 1989).

Numerous other physiological functions appear to be controlled by OP_3 receptors. These include respiration, cardiovascular functions, intestinal transit, feeding, learning and memory, locomotor activity, thermoregulation, hormone secretion, and immune functions, all of which, except hormone secretion, are most often depressed by OP_3 receptor stimulation.

The respiratory depressant effects of OP_3 receptor agonists are thought to result from a decrease in sensitivity of respiratory centers to hypercapnia (see Butelman et al., 1993). They are mediated through OP_3 receptors located both peripherally (Yeadon and Kitchen, 1990) and centrally (Haddad et al., 1984; Morin-Surun et al., 1984) and result from a decrease in volume rather than frequency (Morin-Surun et al., 1984). Similarly, the OP_3 receptors involved in the cardiovascular effects of opioids, which are closely related to their respiratory effects (Hassen et al., 1984b), have both central (Hassen et al., 1984b; Arndt, 1987) and peripheral locations (Randich et al., 1993). This is also true for OP_3 receptors whose stimulation reduces gastrointestinal secretions and motility (Mailman, 1984; Burks et al., 1988; Primi et al., 1988; Kromer, 1989, 1991; Fox-Threlkeld et al., 1994). Depending on the animal species and the ambient temperature, OP_3 receptor agonists can lead to hypothermia or hyperthermia (Adler and Geller, 1988, Handler et al., 1994). The effects of OP_3 receptor stimulation on locomotor activity depend also on the animal species and on the dose of the agonist administered (Bot et al., 1992; Meyer and Meyer, 1993).

III. Molecular Biology of the Opioid Receptors

Three distinct opioid recombinant receptors have been isolated that possess binding and functional properties consistent with their identities as OP_1, OP_2 and OP_3 receptors (see Reisine and Bell, 1993; Kieffer, 1995; Satoh and Minami, 1995). As emphasized above, no sup-

port for the possible existence of subtypes within these receptor classes has been obtained so far from molecular biology investigations. Two variants of the OP_3 receptor, which differ by the presence or the absence of an 8-amino-acid sequence within the C terminal portion of the receptor protein, have been cloned (Bare et al., 1994), but they show similar ligand binding properties and coupling to adenylyl cyclase in transfected CHO-K1 cells. Explanations generally put forward for the subtypes are that they are probably not derived from homologous genes. It should be remembered that single receptor genes can potentially give rise to several pharmacologically distinct receptors, not only via alternative splicing of the primary transcript, as is clearly evident for other receptors (e.g., dopamine D_2 and glutamate receptors), but also by various post-translational modifications, e.g., phosphorylation, palmitoylation, glycosylation, etc. Furthermore, associated proteins can often radically modify pharmacological characteristics as observed in the γ-aminobutyric $acid_A$ ($GABA_A$) receptor family.

The cloning efforts have clearly identified opioid receptors as members of the G protein coupled receptor superfamily with the closest relatives being the somatostatin receptors (Evans et al., 1992; Kieffer et al., 1992). In retrospect, the high homology with the somatostatin receptor family was not unexpected, based upon previous pharmacological studies (Maurer et al., 1982; Pelton et al., 1985). The opioid receptors also have homology with the receptors for angiotensin and for the chemotactic peptides interleukin8 and N-formyl peptide (Evans et al., 1992). A striking structural homology is observed among the three opioid receptor cDNA clones, and the predicted proteins are of similar size (372-amino-acid residues for the OP_1 receptor, 380 for the OP_2 receptor, 398 for the rat and mouse OP_3 receptor). The OP_3 receptor is 66% identical to the OP_1 receptor and 68% identical to the OP_2 receptor, and the two latter receptors share a 58% identity in their respective amino acid sequences (fig. 5).

Within the highly divergent N-terminal extracellular domains, all three opioid receptors have consensus N-linked glycosylation sites; the OP_1 and OP_2 receptors have two such glycosylation sites, whereas the rat OP_3 receptor has five (fig. 1). Variations in the extent of glycosylation result in the mass of the three native opioid receptors being quite different. The transmembrane domains are highly homologous among the three receptors (particularly in the second and third membrane spanning regions, both of which include a negatively charged aspartate residue considered important for function), whereas the C-terminal regions following the postulated cysteine palmitoylation site (positioned shortly after the seventh transmembrane domain) are markedly different. Intracellular protein kinase A and C consensus sites are conserved among the three opioid receptors and, even in the divergent region proximal to the C-terminus, the kinase consensus sites are present in similar locations. The three receptors have a small third intracellular loop of approximately 25 amino acid residues that is probably involved in G protein coupling. This small third intracellular loop contrasts with the large loop found in catecholamine- and muscarinic-receptors, but is characteristic of other peptide receptors, in particular the somatostatin receptors (see Bell and Reisine, 1993). The extracellular loop linking the second and third transmembrane domains is also highly homologous, whereas the second and third extracellular loops are markedly different among the receptors. The overall picture is a family of three opioid receptors displaying somewhat different faces to the extracellular environment with highly conserved operational and regulatory foundations beneath the cell surface.

The pharmacological properties of the three cloned opioid receptors have been investigated, primarily in monkey fibroblast cells (COS) and the Chinese hamster ovary (CHO) cell line. Although agonist inhibition of adenylyl cyclase has been demonstrated for all three opioid receptor clones, detailed functional analysis that would be useful for further characterization of the receptors has not yet been achieved. Presently available pharmacological data have generally been derived only from studies with membranes prepared from transfected non-neuronal cell lines and binding assays under artificial conditions to maximize agonist interactions (i.e., low sodium, no guanosine triphosphate (GTP) or analog). Reisine and his colleagues, who studied the pharmacological profiles of the three recombinant opioid receptors, proposed that the recombinant OP_1, OP_2 and OP_3 receptors may correspond to "δ_2," "κ_1" and "μ_1"-binding sites, respectively (Raynor et al., 1994), but this is still largely speculative as the definitive proof of the existence of OP receptor subtypes has yet to be provided.

A. Cloning of Opioid Receptors

1. OP_1 (δ) receptor clones. Following the initial isolation of a murine OP_1 recombinant receptor from the NG108-15 cell line (Evans et al., 1992; Kieffer et al., 1992), several groups have reported essentially identical sequences from rat and mouse brain (Bzdega et al., 1993 (partial clone); Fukuda et al., 1993; Yasuda et al., 1993; Abood et al., 1994). In addition, a human cDNA encoding a 372-amino-acid protein that has 93% identity with mouse and rat OP_1 receptors has been cloned (Knapp et al., 1994).

Collating the binding data from studies on transfected cells reveals a fairly consistent picture compatible with these clones encoding the OP_1 receptor. For the alkaloids, the recombinant receptor in CHO or COS cells has the following rank order of affinity: NTI (fig. 2, compound 9) > diprenorphine > etorphine > bremazocine (fig. 3, compound 16) ≫ naloxone (fig. 1, compound 3) > morphine (fig. 1, compound 1) > U-50,488 (fig. 3, com-

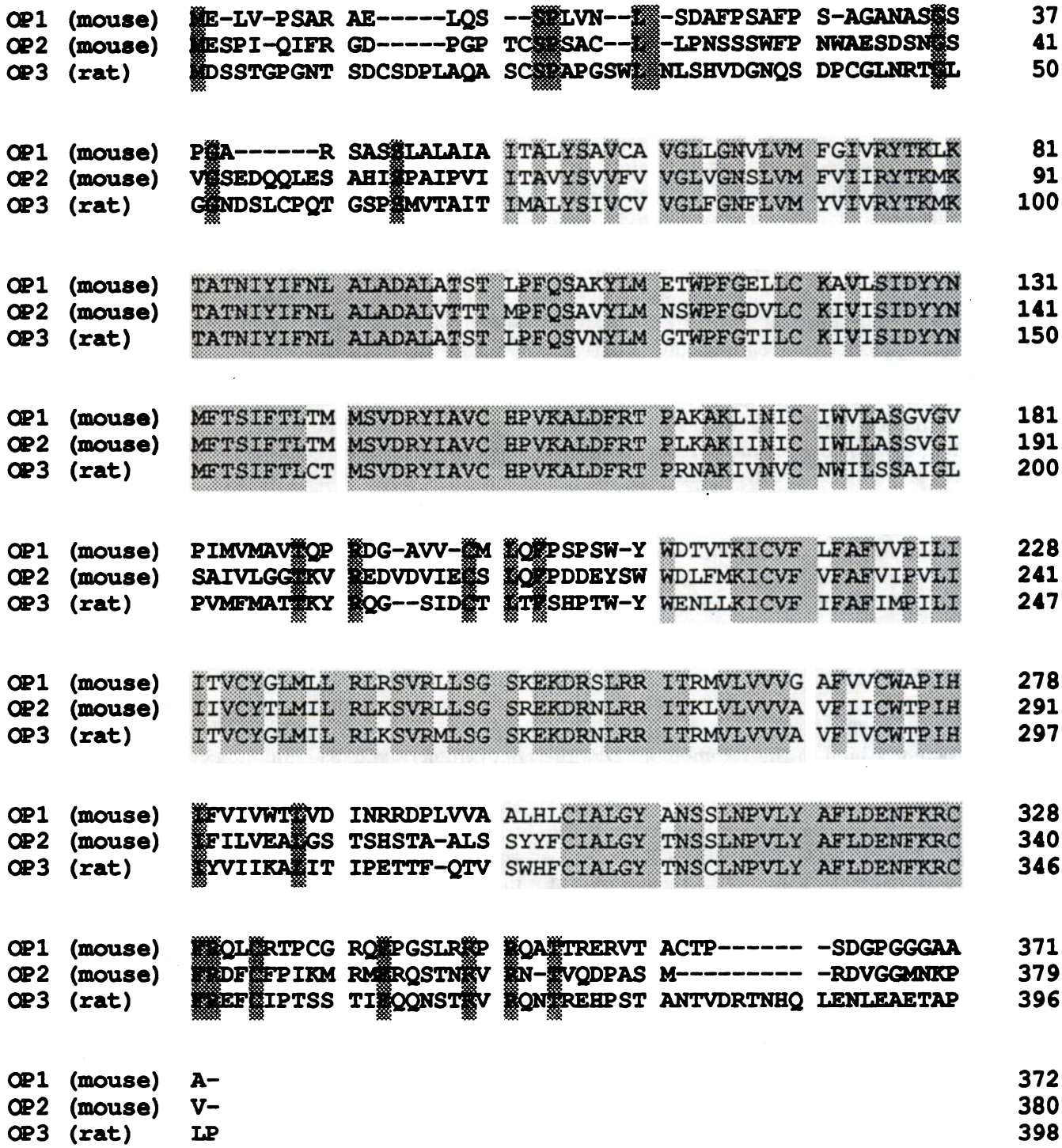

FIG. 5. Comparison of the amino acid sequences of OP_1, OP_2 and OP_3 receptors. The sequences of mouse OP_1, mouse OP_2 and rat OP_3 receptors are shown using the single letter abbreviations of the amino acids. Residues that are identical in at least two of these receptors are enclosed in grey boxes. Gaps introduced to generate this alignment are represented by dashes. The potential sites for N-linked glycosylation in the extracellular domains of these proteins are: mouse OP_1 receptor: Asn(N) 18 and 33; mouse OP_2 receptor: Asn 25 and 39; rat OP_3 receptor: Asn 9, 31, 38, 46 and 53.

pound 18). For the peptide ligands, the rank order is: DTLET > DADLE > TIPP > DPDPE > DAMGO > morphiceptin. The binding data and antagonism of agonist inhibition of adenylyl cyclase by naltriben (fig. 2, compound 12)—a "δ_2"-selective antagonist—and BNTX (fig. 2, compound 10)—a "δ_1" selective antagonist—suggest that the OP_1 recombinant receptor has a pharmacological profile close to that of the so-called "δ_2" binding site (Kong et al., 1993; Raynor et al., 1994).

2. OP_2 (κ) receptor clones. Several essentially identical cDNA clones have been independently isolated and characterized as encoding the OP_2 receptor from mouse (Ya-

suda et al., 1993), rat (Chen et al., 1993b; Li et al., 1993; Meng et al., 1993; Minami et al., 1993; Nishi et al., 1993) and guinea pig (Xie et al., 1994). Dynorphin and its analogs potently bind to the recombinant receptor. In contrast, enkephalin and β-endorphin have low potency in interacting with this receptor. The recombinant receptor, transiently expressed in COS cells, binds alkaloid ligands with the following rank order of affinity: bremazocine (compound 16) > ethylketocyclazocine (compound 15) > U-50,488 (compound 18) > naloxone (compound 3) > levorphanol > naltrindole (compound 9) > morphine (compound 1), and for the peptide ligands: dynorphin A ≫ β-endorphin 1-31 > DPDPE > DAMGO. Comparison of published values revealed relatively large differences in the affinities of prodynorphin-derived opioid peptides, especially α-neoendorphin, from one laboratory to another (Meng et al., 1993; Yasuda et al., 1993). Whether these discrepancies reflect true species differences or methodological variations is unclear at present. Based on the high affinity for U-50,488 (compound 18) and U-69,593 (compound 19), the cloned OP_2 receptor has been proposed to be identical with the so-called "κ_1" binding site (Meng et al., 1993; Yasuda et al., 1993; Lai et al., 1994; Raynor et al., 1994).

3. OP_3 (μ) receptor clones. The OP_3 receptor has been cloned from the rat (Bunzow et al., 1993; Chen et al., 1993a; Fukuda et al., 1993; Thompson et al., 1993; Minami et al., 1994; Zastawny et al., 1994) and from human (Wang et al., 1993, 1994b). Both enkephalin and β-endorphin potently bind to the recombinant OP_3 receptor, whereas this receptor has much less affinity for dynorphin. Clinically used opioids such as morphine (compound 1), methadone, codeine and fentanyl potently and specifically bind to the recombinant OP_3 receptor (but interact with the recombinant OP_2 receptor only at micromolar concentrations). The recombinant rat OP_3 receptor expressed in CHO or COS cells has a rank order of affinity for alkaloid ligands as follows: bremazocine (compound 16) > ethylketocyclazocine (compound 15) > naloxonazine (compound 39) > naloxone (compound 3) > morphine (compound 1) ≫> U-50,488 (compound 18), and for peptide ligands: DAMGO > DADLE > DSLET > DPDPE. These binding data are consistent with the known pharmacological profile of OP_3 receptors (fig. 4).

Most of the compounds have similar affinity for the human and the rat OP_3 receptors. However, the affinities of morphine (compound 1), methadone and codeine are significantly higher for the human OP_3 receptor than for the rat OP_3 receptor (Raynor et al., 1995). With regard to postulated subtypes of "μ" binding sites, the high affinity of naloxonazine (compound 39) for the recombinant OP_3 receptor (Wang et al., 1993; Raynor et al., 1994) would be compatible with its identity with the so-called "μ_1" subtype (Itzhak, 1988).

4. Chimeric opioid receptors. To further investigate the regions of the OP_1 and OP_2 receptors that bind agonists and antagonists, Kong et al. (1994) have generated chimeric OP_1/OP_2 receptors, in which the N-termini of receptors were exchanged to create an OP_2[1-78]OP_1[70-372] receptor and an OP_1[1-69]OP_2[79-380] receptor. The OP_1 receptor selective agonist [^{3}H]DPDPE and antagonist [^{3}H]naltrindole bound to the OP_2[1-78]OP_1[70-372] chimera and a truncated OP_1[70-732] receptor with similar potency as they bind to the wild OP_1 receptor type. Neither radioligand bound to the OP_1[1-69]OP_2[79-380] receptor. These findings suggest that the N-terminus of the OP_1 receptor is not needed for ligand binding, but that the binding domains of selective OP_1 receptor agonists may be localized to either the second or the third extracellular loops of this receptor, because these are the only other extracellular domains that differ in amino acid sequence from the OP_2 (and OP_3) receptor. The results with these chimeric receptors, together with the findings reported on the OP_1 receptor with the aspartate^{95} mutant, suggest that the agonist and antagonist binding domains are distinct but exhibit some overlapping in the native OP_1 receptor (Kong et al., 1993).

In contrast to the results observed with the OP_1 receptor ligands, OP_2 receptor agonists and antagonists appear to bind to clearly separable sites within the OP_2 receptor. OP_2 receptor antagonists bound to the OP_2[1-78]OP_1[70-372] chimera with similar affinity as they bind to the wild OP_2 receptor type. However, the OP_2 receptor agonists did not bind to this chimera. OP_2 receptor agonists did interact with the OP_1[1-69]OP_2[79-380] chimera and also inhibited cyclic adenosine monophosphate (cAMP) formation in cells expressing this chimera or the truncated OP_2[79-380] receptor. In contrast, OP_2 receptor antagonists did not interact with either of the latter modified receptors. These findings indicate that OP_2 receptor antagonists interact selectively with the N-terminal region of the receptor, whereas agonists are likely to interact with either its second or third extracellular loop (Kong et al., 1994).

A study with six chimeric OP_2/OP_3 receptors revealed that the second extracellular loop and the adjoining C-terminal portion of the fourth transmembrane domain are essential for the high affinity binding of dynorphins to the OP_2 receptor. The third extracellular loop and the sixth and seventh transmembrane helices appear to play an important role in determining the selectivity of nor-BNI (compound 30) for the OP_2 over the OP_3 receptor. In particular, within this region, the amino acid residue Glu^{297} has been shown to be critically involved in the binding of one of the basic nitrogens of nor-BNI (compound 30), thereby conferring κ selectivity (Hjorth et al., 1995). On the other hand, U-50,488 (compound 18) and U-69,593 (compound 19) seem to require the whole OP_2 receptor except the second extracellular loop for their high affinity binding. Thus, the OP_2 receptor has differential binding domains for peptide and non-peptide ligands (Xue et al., 1994). In line with this conclusion, it was shown that human OP_2/OP_3 receptor chimeras have

a high affinity for dynorphins only when they include the OP_2 receptor second extracellular loop, whereas their affinity for U-50,488 (compound 18) remains unchanged, whether this loop is that of the OP_2 or the OP_3 receptor (Wang et al., 1994c).

Studies with chimeric OP_1/OP_3 receptors indicated that differences in the structure around the first extracellular loop are critical for DAMGO to distinguish between OP_1 and OP_3 receptors (Onogi et al., 1995). This region is also (at least partly) involved in the discrimination between OP_1 and OP_3 receptors by other peptidic OP_3 selective ligands, such as dermorphins (table 1) and CTOP (fig. 4), but not by non-peptidic ligands, such as morphine (compound 1) and naloxone (compound 3) (Onogi et al., 1995). By contrast, DAMGO distinguishes between OP_2 and OP_3 receptors at the region around the third extracellular loop, and binding studies indicated that this region is involved in the discrimination between OP_2 and OP_3 receptors by both peptidic and non-peptidic OP_3 selective ligands (Minami et al., 1995). Deletion of the C terminus domain and substitution of amino acids in transmembrane domains allowed the demonstration of the requirement of specific charged residues in transmembrane domains 2, 3 and 6 for agonist recognition and intrinsic activity of the OP_3 receptor, and the modest involvement of extensive portions of N- and C-terminal receptor domains in these processes (Surratt et al., 1994).

B. Other Opioid-Related, Receptor-Like Recombinant Proteins

1. Members of the G protein-coupled receptor superfamily. Although homologous to the three cloned opioid receptors, a receptor that was previously characterized as a G protein-coupled receptor closely related to the neurokinin B receptor does not possess the opioid pharmacological characteristics to clearly belong to the opioid receptor family (Xie et al., 1992). However, it should be recognized that this receptor does bind opioid ligands, albeit at low affinity.

More recently, another protein with the typical features of G protein-coupled receptors has been cloned in several species by low stringency screening of cDNA or genomic libraries from brain tissues with cDNA probes of the opioid receptors. This protein (of 360-370 amino acids, depending on the species) has been called ORL_1, for **O**pioid **R**eceptor-**L**ike protein $_1$, because it exhibits a 50-60% sequence homology as compared with OP_1, OP_2 and OP_3 receptors. However, ORL_1 does not bind opioid ligands, except for dynorphins, which ORL_1 binds with low affinity (Zhang and Yu, 1995), when it is expressed in various cell types (Bunzow et al., 1994; Chen et al., 1994; Fukuda et al., 1994; Mollereau et al., 1994; Wang et al., 1994a; Wick et al., 1994). In situ hybridization histochemistry demonstrated that the messenger ribonucleic acid (mRNA) encoding this protein is present in various regions of the central nervous system in rodents, especially the cerebral cortex, thalamus, habenula, hippocampus, central gray, dorsal raphe nucleus, locus coeruleus and the dorsal horn of the spinal cord. Recently, two groups (Meunier et al., 1995; Reinscheid et al., 1995) isolated a peptide (called nociceptin or orphanin FQ) from brain tissues of various species (rat, mouse, pig, bovine and human) that exhibits a nanomolar potency to inhibit forskolin-induced accumulation of cAMP in cells transfected with the ORL_1 coding sequence. Although nociceptin (Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln) and dynorphin A (table 1) are both heptadecapeptides and share six amino acids in the same positions in their respective sequences, the latter peptide has a considerably lower affinity than nociceptin for ORL_1 (Zhang and Yu, 1995). Indeed, nociceptin clearly derives from another precursor than those of the opioid peptides (Meunier et al., 1995).

2. The peculiar status of OBCAM. The isolation and purification of a protein from bovine brain which selectively binds opioid alkaloid ligands was reported by Cho et al. in 1986. It was named OBCAM for **O**pioid **B**inding **C**ell **A**dhesion **M**olecule. Subsequently, the cDNA coding for this protein was cloned (Schofield et al., 1989). A search in the gene bank databases revealed that OBCAM has significant sequence homologies with several members of the immunoglobulin superfamily (Schofield et al., 1989) but not with authentic OP_1, OP_2 and OP_3 receptors. The question of the possible role of OBCAM in the functioning of the endogenous opioid system is still a matter of debate.

C. Opioid Receptor Genes

The genes encoding OP_1, OP_2 and OP_3 receptors have been characterized, notably in the mouse and in the human. The mouse OP_1 receptor gene (designated Oprdl locus) has been mapped to a single locus on chromosome 4 (locus 4D) using both linkage analysis and in situ mapping (Bzdega et al., 1993; Befort et al., 1994; Kaufman et al., 1994). In the mouse, the OP_2 receptor gene is located on chromosome 1, whereas the OP_3 receptor gene is on chromosome 10 (Giros et al., 1995). In the human genome, the gene encoding the OP_1 receptor is located on chromosome 1 (syntenic with the murine locus 4D, Befort et al., 1994), the gene encoding the OP_2 receptor is on the proximal long arm of chromosome 8 (Yasuda et al., 1994), and the OP_3 receptor gene is on the distal arm of chromosome 6 (Wang et al., 1994b). There is no evidence for multiple genes encoding any of the cloned opioid receptors. With regard to gene structure, all three of the genes appear to have introns shortly following the first and the fourth transmembrane domains, therefore presenting the possibility for protein heterogeneity via alternative splicing (Yasuda et al., 1993; Bare et al., 1994; Min et al., 1994; Pasternak and Standifer, 1995).

D. Opioid Receptor Transcripts

There is evidence for multiple mRNA transcripts encoding the three opioid receptors. Northern blots probed for OP_1 receptor detect two major bands in rodent brain (11 and 8.5 kb in the mouse, and 11 and 4.5 kb in the rat). Northern blots probed for OP_3 receptor give bands of 16 and 10.5 kb in the rat brain and of 13.5, 11, 4.3 and 2.8 kb in the human brain (Fukuda et al., 1993; Yasuda et al., 1993; Delfs et al., 1994; Raynor et al., 1995). Alternative splicing of the OP_2 and OP_3 receptor primary transcripts (within the 5′ untranslated region) probably account for these data. Indeed, the differential effects on morphine-induced analgesia of antisense oligodeoxynucleotides targeting various exons of the OP_3 opioid receptor gene were recently interpreted as reflecting the existence of alternative splicing phenomena (Rossi et al., 1995; Pasternak and Standifer, 1995).

Both Northern analysis and in situ hybridization have provided information on the neuroanatomical distribution of OP_1, OP_2 and OP_3 receptor transcripts. There are no striking mismatches between receptor autoradiography and transcript localization studies that cannot be readily explained by neuronal projections (Bzdega et al., 1993; Keith et al., 1993; Thompson et al., 1993; Wang et al., 1993; Yasuda et al., 1993; De Paoli et al., 1994; Mansour et al., 1994, 1995; Minami et al., 1994; Raynor et al., 1995).

IV. Transduction Mechanisms

The functional coupling of the three opioid receptors with G proteins was firmly established several years ago on the bases that guanine nucleotides diminish the specific binding of agonists and that the latter compounds stimulate GTPase activity in several preparations (see Childers, 1991). The predicted structures of the cloned OP_1, OP_2 and OP_3 receptors clearly confirm that they belong to the superfamily of seven-transmembrane spanning G protein-coupled receptors (see Reisine and Bell, 1993; Uhl et al., 1994; Kieffer, 1995; Satoh and Minami, 1995). Furthermore, OP_1 and OP_3 receptors solubilized from rat cortical membranes have been shown to form stable complexes with one or several variants of G_o (Georgoussi et al., 1995). However, it cannot be completely ruled out that opioids may also act independently of G proteins. In particular, in the mouse brain and vas deferens, the binding of the OP_1 receptor agonist BW 373U86 (compound 6) is not affected by guanine nucleotides (Wild et al., 1993b), and the selective OP_3 receptor agonist DAMGO (fig. 4) modulates a Ca^{2+}-dependent K^+ channel independently of G proteins and kinase-mediated mechanisms in cultured bovine adrenal medullary chromaffin cells (Twitchell and Rane, 1994).

The availability of a given type of cloned opioid receptor expressed in a clonal cell line in the absence of any other opioid receptor type provides a unique system for examining the basic cellular events involved in receptor-effector coupling. However, it has to be pointed out that conclusions of such studies do not necessarily apply to the normal situation, i.e., are not directly relevant to the actual opioid-receptor-G protein and -ion channel interactions responsible for the physiological and pharmacological effects of opioids in vivo. The same remark is also applicable to the data obtained with various tumor cell lines that naturally express opioid receptors.

Stimulation by opioid agonists of the cloned rat and human OP_3 receptors expressed in COS and CHO cells or *Xenopus* oocytes reduces not only forskolin-stimulated adenylyl cyclase activity but also the production of inositol triphosphate, in a naloxone-sensitive manner (Chen et al., 1993a; Johnson et al., 1994; Wang et al., 1994b; Raynor et al., 1995). Similarly, in transfected cells, stimulation of OP_1 receptors decreases the accumulation of cAMP resulting from cell exposure to forskolin (Evans et al., 1992; Kong et al., 1993; Yasuda et al., 1993). In embryonic kidney 293 cells, the inhibition of adenylyl cyclase activity attributable to activation of the cloned OP_2 receptor could involve the G_z subtype of G proteins (Lai et al., 1995). Activation of the mouse, rat and human OP_2 receptors expressed in COS or PC-12 cells also leads to inhibition of cAMP formation (Chen et al., 1993b; Meng et al., 1993; Yasuda et al., 1993; Kong et al., 1994; Tallent et al., 1994; Wang et al., 1994b; Xie et al., 1994). In *Xenopus* oocytes coinjected with β_2-adrenoceptor mRNA and mouse OP_1 receptor mRNA, OP_1 receptor agonists cause a naltrexone-reversible concentration-dependent inhibition of the isoprenaline-induced increase of cAMP production (Tamir and Kushner, 1993).

OP_3 receptor agonists are also able to inhibit adenylyl cyclase activity in tumor cell lines (Frey and Kebabian, 1984; Yu et al., 1986). Similarly, in NG108-15 cells, activation of OP_1 receptors inhibits adenylyl cyclase activity. Although the G protein $G_{\alpha i2}$ seems to be specifically involved in this process (McKenzie and Milligan, 1990), at least two other G proteins ($G_{\alpha o2}$ and one isoform of $G_{\alpha i3}$) can interact with the OP_1 receptors in this and other cell lines (Roerig et al., 1992; Prather et al., 1994). The high-affinity OP_2 receptor that is expressed in mouse thymoma R1.1 cell line is also negatively coupled to adenylyl cyclase through a pertussis toxin-sensitive G protein (Lawrence and Bidlack, 1993).

Studies on brain tissues indicated that stimulation of OP_1 and OP_3 receptors can inhibit adenylyl cyclase activity (Chneiweiss et al., 1988; Polastron et al., 1990). Furthermore, differential blockade by BNTX (compound 10) and naltriben (compound 12) of DPDPE- and [D-Ala^2]deltorphin II-mediated inhibition of adenylyl cyclase activity in rat caudate-putamen has been reported in support of the possible existence of OP_1 receptor subtypes (Noble and Cox, 1995). However, in homogenates of the same brain structure incubated with agents that block the binding of ligands to OP_3 receptors, no change

in opioid-inhibited adenylyl cyclase has been detected (Nijssen et al., 1992). Furthermore, in rat olfactory bulb, selective OP_1 and OP_3, but not OP_2, receptor agonists exert a dual effect on adenylyl cyclase activity that is GTP-dependent and pertussis toxin-sensitive. Thus, opioids increase basal adenylyl cyclase activity but inhibit the enhanced cAMP production attributable to various effectors, possibly through differential actions on the various forms of the enzyme (Onali and Olianas, 1991; Olianas and Onali, 1992, 1994). Contradictory data have been published about the coupling of OP_2 receptors to adenylyl cyclase in guinea pig brain membranes, especially in those prepared from the cerebellum (Konkoy and Childers, 1989, 1993; Polastron et al., 1990). In this region, OP_2 receptors appear to be coupled also to G_{i1}-mediated inhibition of phospholipase C activity (Misawa et al., 1990, 1995).

In *Xenopus* oocytes coexpressing a G protein-activated K^+ channel and the rat OP_3 receptor, DAMGO induced an inwardly rectifying current that was blocked by naloxone, as expected of the functional interaction between the two expressed proteins (Chen and Yu, 1994). Similarly, a functional coupling between the mouse OP_1 receptor and a G protein-activated K^+ channel co-expressed in oocytes was recently demonstrated (Ikeda et al., 1995). Other cation channels can also be controlled by OP_1 receptors, notably in NG108-15 cells, in which Taussig et al. (1992) found that a $G_{\alpha o1}$ subtype of G protein is implicated in the functional coupling of these receptors with a voltage-dependent Ca^{2+} channel. Such multiple coupling potentialities were further illustrated by the data reported by Jin et al. (1994), which showed that OP_1 receptor stimulation in the same hybridoma (neuroblastoma × glioma) cells mobilized Ca^{2+} from inositol triphosphate-sensitive stores, via a pertussis toxin-sensitive G protein.

Co-expression of OP_2 receptors and the BI-type of Ca^{2+} channels ($\alpha 1$ plus β subunits) allowed transfected *Xenopus* oocytes to respond to OP_2 receptor agonists by closure of these channels via a pertussis toxin-sensitive G protein (Kaneko et al., 1994a). Furthermore, in transfected PC-12 cells, the cloned mouse OP_2 receptor appears to inhibit, also in a pertussis toxin-sensitive manner, a N-type Ca^{2+} current (Tallent et al., 1994). Such multiple coupling mechanisms, e.g., with adenylyl cyclase, phospholipase C and various cation channels, probably involved different G proteins, in line with the demonstration by Prather et al. (1995) that, in transfected CHO cells, the cloned OP_2 receptor can directly interact with $G_{\alpha i2}$, $G_{\alpha i3}$ and $G_{\alpha o2}$.

That selective OP_3 receptor agonists can activate inward rectifying K^+ conductance has been reported for various brain regions (Loose and Kelly, 1990; Wuarin and Dudek, 1990; Wimpey and Chavkin, 1991; Chiu et al., 1993). Interestingly, like OP_3 receptors in rat locus coeruleus and hippocampus (Williams and North, 1984; Wimpey and Chavkin, 1991) and OP_1 receptors on guinea pig peripheral neurons (Mihara and North, 1986), OP_2 receptors can also increase K^+ conductance, at least in neurons of the substantia nigra in the latter species (Grudt and Williams, 1993).

An OP_3 receptor-mediated reduction of neuronal Ca^{2+} current has also been found in various preparations. Diverse Ca^{2+} channels, particularly the N-type, appear to be involved in this response, and their coupling to the OP_3 receptors occurs via a pertussis toxin-sensitive G_o subclass of G proteins (Moises et al., 1994a, b; Rhim and Miller, 1994). Like OP_3 receptors, OP_2 receptors in rat dorsal root ganglion sensory neurons are also negatively coupled to several pharmacologically distinct types of Ca^{2+} channels, including probably the N-type (Moises et al., 1994b).

A coupling of opioid receptors with G_s-proteins responsible for excitatory effects of opioid agonists on target cells has also been hypothesized (see Crain and Shen, 1990; Shen and Crain, 1990; Gintzler and Xu, 1991). These so-called "excitatory" opioid receptors would be activated by lower concentrations of opioids than the "inhibitory" receptors coupled to G_o or G_i proteins (see Crain and Shen, 1990; Wang and Gintzler, 1994). However, recent investigations with transfected cells clearly demonstrated that opioid receptors can couple with various G_i and G_o proteins, and also G_z, but not with G_s. Indeed, in *Xenopus* oocytes expressing the rat OP_2 receptor, the selective OP_2 receptor agonist U-50488 (compound 18) stimulates cAMP production and mobilizes intracellular Ca^{2+} through the positive coupling of the receptor to both adenylyl cyclase and phospholipase C, via pertussis toxin-sensitive G proteins (G_i, G_o; Kaneko et al., 1994b). The increased cAMP production attributable to opioid receptor stimulation results in fact from the activation of type II adenylyl cyclase via the $\beta\gamma$ subunits of G proteins (Chan et al., 1995; Tsu et al., 1995).

V. Concluding Remarks

Major advances have been made in the understanding of opioid receptors from stereospecific binding in 1971 to receptor cloning in 1992. The three opioid receptor types identified on the basis of biochemical and pharmacological evidence have thus been cloned. The recombinant receptors exhibit characteristics similar to those of the native receptors. However, the recombinant receptors are currently available from a few animal species only. Information about subtypes of these receptors is still in its infancy, partly because of unavailability of highly selective agonists and antagonists. To date, molecular biology data have not yet provided support to the possible existence of OP receptor subtypes such as those suspected from pharmacological observations. Clearly, much more must be done to answer the pending question of the presence or the absence of OP receptor subtypes in the central and peripheral nervous systems.

Very little is known to date regarding the molecular mechanisms (phosphorylation, internalization, control of opioid receptor gene expression, etc.) involved in the regulation of opioid receptor functioning. Whether such mechanisms contribute to tolerance and dependence phenomena is also a matter of debate and should be investigated further using molecular biology approaches. The construction of opioid receptor chimeras and site-directed mutagenesis already pointed to amino acids critically involved in the binding of agonists and antagonists onto opioid receptors, but much more has yet to be done to really assess the physicochemical features of the interaction of opioids with their receptors. Knowledge of these features is probably the key for the synthesis of potent and selective agonists and antagonists as pharmacological tools and therapeutic agents. Finally, antisense strategy and direct alterations (transgenesis, knock-out by conditional homologous recombination, etc.) of the genes encoding opioid receptors can be expected to generate new in vivo models for assessing further the various physiological and pathophysiological implications of these receptors.

Acknowledgements. The authors thank Mrs. C. Sais for excellent secretarial assistance and Professor B. P. Roques, Professor E. J. Simon, Professor J. L. Henry, Dr. M. M. Dhar and the other members of the IUPHAR subcommittee on Opioid Receptors for helpful discussions during preparation of this manuscript.

REFERENCES

ABOOD, M. E., NOEL, M. A., FARNWORTH, J. S., AND TAO, Q.: Molecular cloning and expression of a delta opioid receptor from rat brain. J. Neurosci. Res. **37:** 714-719, 1994.

ADLER, M. W., AND GELLER, E. B.: The opioid system and temperature regulation. Annu. Rev. Pharmacol. Toxicol. **28:** 429-449, 1988.

AKIYAMA, K., GEE, K. W., MOSBERG, H. I., HRUBY, V. J., AND YAMAMURA, H. I.: Characterization of [^{3}H][2-D-penicillamine, 5-D-penicillamine]-enkephalin binding to δ opiate receptors in the rat brain and neuroblastoma-glioma hybrid cell line (NG 108-15). Proc. Natl. Acad. Sci. USA **82:** 2532-2537, 1985.

AMICHE, M., SAGAN, S., MOR, A., DELFOUR, A., AND NICOLAS, P.: Dermenkephalin (Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2): a potent and fully specific agonist for the δ opioid receptor. Mol. Pharmacol. **35:** 774-779, 1989.

ARNDT, J. O.: Opiate receptors in the CNS and their possible role in cardiovascular control. *In* Brain Peptides and Catecholamines in Cardiovascular Regulation, ed. by J. P. Buckley and C. M. Ferrario, pp. 137-151, Raven Press, New York, 1987.

ARVIDSSON, U., DADO, R. J., RIEDL, M., LEE, J. H., LAW, P. Y., LOH, H. H., ELDE, R., AND WESSENDORF, M. W.: δ-opioid receptor immunoreactivity: distribution in brain stem and spinal cord, and relationship to biogenic amines and enkephalin. J. Neurosci. **15:** 1215-1235, 1995.

AUDIGIER, Y., ATTALI, B., MAZARGUIL, H., AND CROS, J.: Characterisation of [^{3}H]etorphine binding in guinea pig striatum after blockade of μ and δ sites. Life Sci. **31:** 1287-1290, 1982.

BAAMONDE, A., DAUGÉ, V., RUIZ-GAYO, M., FULGA, I. G., TURCAUD, S., FOURNIÉ-ZALUSKI, M. C. AND ROQUES, B. P.: Antidepressant-type effects of endogenous enkephalins protected by systemic RB 101 are mediated by opioid delta and dopamine D1 receptor stimulation. Eur. J. Pharmacol. **216:** 157-166, 1992.

BARBER, A., BARTOSZYK, G. D., BENDER, H. M., GOTTSCHLICH, R., GREINER, H. E., HARTING, J., MAULER, F., MINCK, K. O., MURRAY, R. D., AND SEYFRIED, C. A.: A pharmacological profile of the novel, peripherally-selective κ-opioid receptor agonist, EMD 61753. Br. J. Pharmacol. **113:** 1317-1327, 1994a.

BARBER, A., BARTOSZYK, G. D., GREINER, H. E., MAULER, F., MURRAY, R. D., SEYFRIED, C. A., SIMON, M., BOTTSCHLICH, R., HARTING, J., AND LUES, I.: Central and peripheral actions of the novel κ-opioid receptor agonist, EMD 60400. Br. J. Pharmacol. **111:** 843-851, 1994b.

BARE, L. A., MANSSON, E., AND YANG, D. M.: Expression of two variants of the human mu opioid receptor mRNA in SK-N-SH cells and human brain. FEBS Lett. **354:** 213-216, 1994.

BAUSCH, S. S., PATTERSON, T. A., APLLEYARD, S. M., AND CHAVKIN, C.: Immunocytochemical localization of delta opioid receptor in mouse brain. J. Chem. Neuroanat. **8:** 175-189, 1995.

BECKETT, A. H., AND CASY, A. F.: Synthetic analgesics: stereochemical considerations. J. Pharm. Pharmacol. **6:** 986-1001, 1954.

BEFORT, K., MATTEI, M. G., ROECKEL, N., AND KIEFFER, B.: Chromosomal localization of the delta opioid receptor gene to human 1p 34,3- p36.1 and mouse 4D bands by in situ hybridization. Genomics **20:** 143-145, 1994.

BELL, G., AND REISINE, T.: Molecular Biology of somatostatin receptors. Trends Neurosci. **16:** 34-38, 1993.

BESSE, D., LOMBARD, M. C., ZAJAC, J. M., ROQUES, B. P., AND BESSON, J. M.: Pre- and postsynaptic distribution of mu, delta and kappa opioid receptors in the superficial layers of the dorsal horn of the rat spinal cord. Brain Res. **521:** 15-22, 1990.

BILSKY, E. J., CALDERON, S. N., WANG, T., BERNSTEIN, R. N., DAVIS, P., HRUBY, V. J., MCNUTT, R. W., ROTHMAN, R. B., RICE, K. C., AND PORRECA, F.: SNC 80, a selective, nonpeptidic and systemically active opioid delta agonist. J. Pharmacol. Exp. Ther. **273:** 359-366, 1995.

BIRCH, P. J., HAYES, A. G., SHEEHAN, M. J., AND TYERS, M. B.: Nor-binaltorphimine: antagonistic profile at κ opioid receptors. Eur. J. Pharmacol. **144:** 405-408, 1987.

BLANCHARD, S. G., LEE, P. H. K., PUGH, W. W., AND CHANG, K. J.: Characterization of the binding of a morphine (μ) receptor specific ligand: Tyr-Pro-NMePhe-D-Pro-NH_2, [^{3}H]-PL17. Mol. Pharmacol. **31:** 326-333, 1987.

BOT, G., CHAHL, L. A., BRENT, P. J., AND JOHNSTON, P. A.: Effects of intracerebroventricularly administered mu-, delta- and kappa-opioid agonists on locomotor activity of the guinea pig and the pharmacology of the locomotor response to U50,488H. Neuropharmacology **31:** 825-833, 1992.

BOURGOIN, S., BENOLIEL, J. J., COLLIN, E., MAUBORGNE, A., POHL, M., HAMON, M., AND CESSELIN, F.: Opioidergic control of the spinal release of neuropeptides: possible significance for the analgesic effects of opioids. Fundam. Clin. Pharmacol. **8:** 307-321, 1994.

BOWEN, W. D., HELLEWELL, S. B., KELEMEN, M., HUEY, R., AND STEWARD, D.: Affinity labelling of delta-opiate receptors using [D-Ala^2, Leu^5, Cys^6]enkephalin: covalent attachment via thiol-disulfide exchange. J. Biol. Chem. **262:** 13434-13439, 1987.

BOYLE, S. J., MEECHAM, K. G., HUNTER, J. C., AND HUGHES, J.: [^{3}H]-CI-977: a highly selective ligand for the κ-opioid receptor in both guinea-pig and rat forebrain. Mol. Neuropharmacol. **1:** 23-29, 1990.

BRADBURY, A. F., SMYTH, D. G., SNELL, C. R., BIRDSALL, N. J. M., AND HULME, E. C.: C-fragment of lipoprotein has a high affinity for brain opiate receptors. Nature (Lond.) **260:** 793-795, 1976.

BROCCARDO, M., AND IMPROTA, G.: Antidiarrheal and colonic antipropulsive effects of spinal and supraspinal administration of the natural δ opioid receptor agonist, [D-Ala^2]deltorphin II, in the rat. Eur. J. Pharmacol. **218:** 69-73, 1992.

BROWNSTEIN, M. J.: A brief history of opiates, opioid peptides, and opioid receptors. Proc. Natl. Acad. Sci. USA **90:** 5391-5393, 1993.

BUNZOW, J. R., GRANDY, D. K., AND KELLY, M.: Cloning, pharmacological characterization and distribution of a rat mu-opioid receptor: GenBank Accession No. U02083, 1993.

BUNZOW, J. R., SAEZ, C., MORTRUD, M., BOUVIER, C., WILLIAMS, J. T., LOW, M., AND GRANDY, D. K.: Molecular cloning and tissue distribution of a putative member of the rat opioid receptor gene family that is not a μ, δ or κ opioid receptor type. FEBS Lett. **347:** 284-288, 1994.

BURKS, T. F., FOX, D. A., HIRNING, L. D., SHOOK, J. E., AND PORRECA, F.: Regulation of gastrointestinal function by multiple opioid receptors. Life Sci. **43:** 2177-2181, 1988.

BUTELMAN, E. R., FRANCE, C. P., AND WOODS, J. H.: Apparent pA2 analysis on the respiratory depressant effects of alfentanil, etonitazene, ethylketocyclazocine (EKC) and Mr2033 in rhesus monkeys. J. Pharmacol. Exp. Ther. **264:** 145-151, 1993.

BUZAS, B., TOTH, G., CAVAGNERO, S., HRUBY, V. J., AND BORSODI, A.: Synthesis and binding characteristics of the highly delta specific new tritiated opioid peptide, [^{3}H]deltorphin II. Life Sci. **50:** PL75–PL78, 1992.

BZDEGA, T., CHIN, H., KIM, H., JUNG, H. H., KOZAK, C. A., AND KLEE W. A.: Regional expression and chromosomal localization of the δ opiate receptor gene. Proc. Natl. Acad. Sci. USA **90:** 9305-9309, 1993.

CHAILLET, P., COULAUD, A., ZAJAC, J. M., FOURNIÉ-ZALUSKI, M. C., COSTENTIN, J., AND ROQUES, B. P.: The μ rather than the δ subtype of opioid receptors appears to be involved in enkephalin induced analgesia. Eur. J. Pharmacol. **101:** 83-90, 1984.

CHAN, J. S. C., CHIU, T. T., AND WONG, Y. H.: Activation of type II adenylyl cyclase by the cloned μ-opioid receptor: coupling to multiple G proteins. J. Neurochem. **65:** 2682-2689, 1995.

CHANG, A. C., TAKEMORI, A. E., OJALA, W. H., GLEASON, W. B., PORTOGHESE, P. S.: κ opioid receptor selective affinity labels: electrophilic benzeneacetamides as κ-selective opioid antagonists. J. Med. Chem. **37:** 4490-4498, 1994.

CHANG, K. J., KILLIAN, A., HAZUM, E., CUATRECASAS, P., AND CHANG, J. K.: Morphiceptin (NH_4-Tyr-Pro-Phe-Pro-$CONH_2$): a potent and specific agonist for morphine (mu) receptors. Science (Wash. DC) **212:** 75-77, 1981.

CHANG, K. J., WEI, E. T., KILLIAN, A., AND CHANG, J. K.: Potent morphiceptin analogs: structure activity relationships and morphine like activities. J. Pharmacol. Exp. Ther. **227:** 403-408, 1983.

CHANG, K. J., RIGDON, G. C., HOWARD, J. L., AND MCNUTT, R. W.: A novel, potent and selective nonpeptidic delta opioid receptor agonist BW373U86. J. Pharmacol. Exp. Ther. **267:** 852-857, 1993.

CHAVKIN, C., JAMES, I. F., AND GOLDSTEIN, A.: Dynorphin is a specific endogenous ligand of the κ opioid receptor. Science (Wash. DC) **215:** 413-415, 1982.

CHEN, Y., FAN, Y., LIU, L., MESTEK, A., TIAN, M. T., KOZAK, C. A., AND YU, L.: Molecular cloning, tissue distribution and chromosomal localization of a novel member of the opioid receptor gene family. FEBS Lett. **347:** 279-283, 1994.

CHEN, Y., MESTEK, A., LIU, J., HURLEY, J. A., AND YU, L.: Molecular cloning and functional expression of a mu opioid receptor from rat brain. Mol. Pharmacol. **44:** 8-12, 1993a.

CHEN, Y., MESTEK, A., LIU, J., AND YU, L.: Molecular cloning of a rat kappa opioid receptor reveals sequence similarities to the mu and delta opioid receptors. Biochem. J. **295:** 625-628, 1993b.

CHEN Y., AND YU, L.: Differential regulation by cAMP-dependent protein kinase and protein kinase C of the μ opioid receptor coupling to a G protein-activated K^+ channel. J. Biol. Chem. **269:** 7839-7842, 1994.

CHENG, P. Y., SVINGOS, A. L., WANG, H., CLARKE, C. L., JENAB, S., BECZKOWSKA, I. W., INTURRISI, C. E., AND PICKEL, V. M.: Ultrastructural immunolabeling shows prominent presynaptic vesicular localisation of δ-opioid receptor within both enkephalin- and nonenkephalin-containing axon terminals in the superficial layers of the rat cervical spinal cord. J. Neurosci. **15:** 5976-5988, 1995.

CHILDERS, S. R.: Opioid receptor-coupled second messengers systems. Life Sci. **48:** 1991-2003, 1991.

CHIU, T. H., YEH, M. H., TSAI, S. K., AND MOK, M. S.: Electrophysiological actions of alfentanil: intracellular studies in the rat locus coeruleus neurones. Br. J. Pharmacol. **110:** 903-909, 1993.

CHNEIWEISS, H., GLOWINSKI, J., AND PRÉMONT, J.: Mu and delta opiate receptors coupled negatively to adenylate cyclase on embryonic neurons from mouse striatum in primary culture. J. Neurosci. **8:** 3376-3382, 1988.

CHO, T. M., HASEGAWA, J., GE, B. L., AND LOH, H. H.: Purification to apparent homogeneity of a mu specific opioid receptor from brain. Proc. Natl. Acad. Sci. USA **83:** 4138-4142, 1986.

CHOI, H., MURRAY, T. F., DELANDER, G. E., CALDWELL, V., AND ALDRICH, J. V.: N-terminal alkylated derivatives of [D-Pro^{10}]dynorphin A-(1-11) are highly selective for κ-opioid receptors. J. Med. Chem. **35:** 4638-4639, 1992.

CLARK, C. R., BIRCHMORE, B., SHARIF, N. A., HUNTER, J. C., HILL, R. G., AND HUGHES, J.: PD 117302: a selective agonist for the κ-opioid receptors. Br. J. Pharmacol. **93:** 618-626, 1988.

CLARK, J. A., LIU, L., PRICE, M., HERSH, B., EDELSON, M., AND PASTERNAK, G. W.: Kappa opiate receptor multiplicity: evidence for two U50,488-sensitive K1 subtypes and a novel k3 subtype. J. Pharmacol. Exp. Ther. **251:** 461-468, 1989.

COHEN, M. L., SHUMAN, R. T., OSBORNE, J. J., AND GESELLCHEN, P. D.: Opioid agonist activity of ICI 174864 and its carboxypeptidase degradation product, LY281217. J. Pharmacol. Exp. Ther. **238:** 769-772, 1986.

COMER, S. D., HOENICKE, E. M., SABLE, A. I., MCNUTT, R. W., CHANG, K. J., DE COSTA, B. R., MOSBERG, H. I., AND WOODS, J. H.: Convulsive effects of systemic administration of the delta opioid agonist BW373U86 in mice. J. Pharmacol. Exp. Ther. **267:** 888-895, 1993.

CONTRERAS, P. C., TAM, L., DROWER, E., AND RAFFERTY, M. F.: [^{3}H]naltrindole: a potent and selective ligand for labeling δ-opioid receptors. Brain Res. **604:** 160-164, 1993.

CORBETT, A. D., PATERSON, S. J., MCKNIGHT, A. T., MAGNAN, J., AND KOSTERLITZ, H. W.: Dynorphin1–8 and dynorphin1–9 are ligands for the κ-subtype of opioid receptor. Nature (Lond.) **299:** 79-81, 1982.

COSTELLO, G. F., MAIN, B. G., BARLOW, J. J., CARROLL, J. A., AND SHAW, J. S.: A novel series of potent and selective agonists at the opioid kappa-receptor. Eur. J. Pharmacol. **151:** 475-478, 1988.

COTTON, R., GILES, M. B., MILLER, L., SHAW, J. S., AND TIMMS, D.: ICI 174,864: a highly selective antagonist for the δ opioid receptor. Eur. J. Pharmacol. **97:** 331-332, 1984.

COX, B. M., GOLDSTEIN, A., AND LI, C. H.: Opioid activity of a peptide, beta-endorphin (61-91), derived from beta-lipotropin. Proc. Natl. Acad. Sci. USA **73:** 1821-1823, 1976.

CRABTREE, B. L.: Review of naltrexone, a long-acting opiate antagonist. Clin. Pharmacol. **3:** 273-280, 1984.

CRAIN, S. M., AND SHEN, K. F.: Opioids can evoke direct receptor-mediated excitatory effects on sensory neurons. Trends Pharmacol. Sci. **11:** 77-81, 1990.

CRUCIANI, R. A., LUTZ, R. A., MUNSON, P. J., AND RODBARD, D.: Naloxonazine effect on the interaction of enkephalin analogs with mu-1, mu-2 and delta opioid binding sites in rat brain membranes. J. Pharmacol. Exp. Ther. **242:** 15-20, 1987.

DADO, R. J., LAW, P. Y., LOH, H. H., AND ELDE, R.: Immunofluorescent identification of a delta (δ)-opioid receptor on primary afferent nerve terminals. Neuroreport **5:** 341-344, 1993.

DE COSTA, B. R., BAND, L., ROTHMAN, R. B., JACOBSON, A. E., BYKOV, V., PERT, A., AND RICE, K. C.: Synthesis of an affinity ligand ("UPHIT") for in vivo acylation of the κ-opioid receptor. FEBS Lett. **249:** 178-182, 1989.

DELAY-GOYET, P., SEGUIN, C., GACEL, G., AND ROQUES, B. P.: [^{3}H][D-Ser^2(O-tert-butyl), Leu^5]enkephalyl-Thr^6 and [D-Ser^2 (O-tert-butyl), Leu^5]enkephalyl-Thr^6 (O-tert-butyl): two new enkephalin analogs with both a good selectivity and a high affinity toward delta-opioid binding sites. J. Biol. Chem. **263:** 4124-4130, 1988.

DELAY-GOYET, P., ZAJAC, J. M., RIGAUDY, P., FOUCAUD, B., AND ROQUES, B. P.: Comparative binding properties of linear and cyclic delta- selective enkephalin analogues: [^{3}H]-[D-Thr^2, Leu^5] enkephalyl-Thr^6 and [^{3}H]-[D-Pen^2, D-Pen^5] enkephalin. FEBS Lett. **183:** 439-443, 1985.

DELAY-GOYET, P., ZAJAC, J. M., AND ROQUES, B. P.: Improved quantitative radioautography of rat brain δ-opioid binding sites using [^{3}H]DSTBULET, a new highly potent and selective linear enkephalin analogue. Neurochem. Int. **16:** 341-368, 1990.

DELFS, J., KONG, H., YU, L., REISINE, T., AND CHESSELET, M. F.: Expression of the mu opioid receptor mRNA in rat brain: an in situ hybridization study at the single cell level. J. Comp. Neurol. **345:** 46-68, 1994.

DEPAOLI, A. M., HURLEY, K. M., YASADA, K., REISINE, T., AND BELL, G.: Distribution of κ opioid receptor mRNA in adult mouse brain: an in situ hybridization histochemistry study. Mol. Cell. Neurosci. **5:** 327-335, 1994.

DROWER, E. J., STAPELFELD, A., RAFFERTY, M. F., DE COSTA, B. R., RICE, K. C., AND HAMMOND, D. L.: Selective antagonism by naltrindole of the antinociceptive effects of the delta opioid agonist cyclic[D-Penicillamine^2-D-Penicillamine^5] enkephalin in the rat. J. Pharmacol. Exp. Ther. **259:** 725-731, 1991.

DUPIN, S., TAFANI, J. A. M., MAZARGUIL, H., AND ZAJAC, J. M.: [^{125}I][D-Ala^2]deltorphin-I: a high affinity, delta selective opioid receptor ligand. Peptides **12:** 825-830, 1991.

EMMERSON, P. J., LIU, M. R., WOODS, J. H., AND MEDZIHRADSKY, F.: Binding affinity and selectivity of opioids at mu, delta and kappa receptors in monkey brain membranes. J. Pharmacol. Exp. Ther. **271:** 1630-1637, 1994.

ERSPAMER, V., MELCHIORRI, P., FALCONIERI ERSPAMER, G., NEGRI, L., CORSI, R., SEVERINI, C., BARRA, D., SIMMACO, M., AND KREIL, G.: Deltorphins: a family of naturally occurring peptides with high affinity and selectivity for δ opioid binding sites. Proc. Natl. Acad. Sci. USA **86:** 5188-5192, 1989.

EVANS, C. J., KEITH, D. E., JR., MORRISON, H., MAGENDZO, K., AND EDWARDS, R. H.: Cloning of a delta opioid receptor by functional expression. Science (Wash. DC) **258:** 1952-1955, 1992.

FANG, F. G., FIELDS, H. L., AND LEE, N. M.: Action at the mu receptor is sufficient to explain the supraspinal effects of opiates. J. Pharmacol. Exp. Ther. **238:** 1039-1044, 1986.

FANG, L., KNAPP, R. J., HORVATH, R., MATSUNAGA, T. O., HAASETH, R. C., HRUBY, V. J., PORRECA, F., AND YAMAMURA, H. I.: Characterization of [^{3}H] naltrindole binding to delta opioid receptors in mouse brain and mouse vas deferens: evidence for delta opioid receptor heterogeneity. J. Pharmacol. Exp. Ther. **268:** 836-846, 1994.

FANG, L. F., KNAPP, R. J., MATSUNAGA, T., WEBER, S. J., DAVIS, T., HRUBY, V. J., AND YAMAMURA, H. I.: Synthesis of [D-Ala^2, 4′-^{125}I-Phe^3, Glu^4]deltorphin and characterization of its δ opioid receptor binding properties. Life Sci. **51:** PL189–PL193, 1992.

FITZGERALD, L. W., AND TEITLER, M.: Quantitative autoradiographic analysis of [^{3}H]carfentanyl binding to mu opiate receptors in rat brain. Synapse **14:** 154-159, 1993.

FOWLER, C. J., AND FRASER, G. L.: μ-, δ-, κ-opioid receptors and their subtypes. A critical review with emphasis on radioligand binding experiments. Neurochem. Int. **24:** 401-426, 1994.

FOX-THRELKELD, J. A. E. T., DANIEL, E. E., CHRISTINCK, F., HRUBY, V. J., CIPRIS, S., AND WOSKOWSKA, Z.: Identification of mechanisms and sites of action of mu and delta receptor activation in the canine intestine. J. Pharmacol. Exp. Ther. **268:** 689-700, 1994.

FRANCE, C. P., AND WOODS, J. H.: Naloxone benzoylhydrazone is a μ-selective opioid antagonist without κ agonist effects in rhesus monkeys. Behav. Pharmacol. **3:** 133-141, 1992.

FRANCE, C. P., MEDZIHRADSKY, F., AND WOODS, J. H.: Comparison of kappa opioids in rhesus monkeys: behavioral effects and receptor binding affinities. J. Pharmacol. Exp. Ther. **268:** 47-58, 1994.

FREY, A., AND KEBABIAN, J. W.: μ-opiate receptors in 7315c tumor tissue mediate inhibition of immunoreactive prolactin release and adenylate cyclase activity. Endocrinology **115:** 1797-1804, 1984.

FREYE, E., SCHNITZLER, M., AND SCHENK, G.: Opioid-induced respiratory depression and analgesia may be mediated by different subreceptors. Pharm. Res. **8:** 196-199, 1991.

FUKUDA, K., KATO, S., MORI, K., IWABE, N., MIYATA, T., NISHI, M., AND TAKESHIMA, H.: Primary structures and expression from cDNAs of rat opioid receptor delta and mu-subtypes. FEBS Lett. **327:** 311-314, 1993.

FUKUDA, K., KATO, S., MORI, K., NISHI, M., TAKESHIMA, H., IWABE, N., MIYATA, T., HOUTANI, T., AND SUGIMOTO, T.: cDNA cloning and regional distribution of a novel member of the opioid receptor family. FEBS Lett. **343:** 42-46, 1994.

GACEL, G., FOURNIÉ-ZALUSKI, M. C., AND ROQUES, B. P.: D-Tyr-Ser-Gly-Phe-Leu-Thr, a highly preferential ligand for delta opiate receptors. FEBS Lett. **118:** 245-247, 1980.

GACEL, G., ZAJAC, J. M., DELAY-GOYET, P., DAUG., V., AND ROQUES, B. P.: Investigation of the structural parameters involved in the mu and delta opioid receptor discrimination of linear enkephalin-related peptides. J. Med. Chem. **31:** 374-383, 1988.

GACEL, G. A., FELLION, E., BAAMONDE, A., DAUG., V., AND ROQUES, B. P.: Synthesis, biochemical and pharmacological properties of BUBUC, a highly selective and systemically active agonist for in vivo studies of delta-opioid receptors. Peptides **11:** 983-988, 1990.

GAIRIN, J. E., GOUARDERES, C., MAZARGUIL, H., ALVINERIE, P., AND CROS, J.:

[D-Pro^{10}] dynorphin-(1-11) is a higly potent and selective ligand for κ opioid receptors. Eur. J. Pharmacol. **106:** 457-458, 1985.

GEORGOUSSI, Z., MILLIGAN, G., AND ZIOUDROU, C.: Immunoprecipitation of opioid receptor-Go-protein complexes using specific GTP binding-protein antisera. Biochem. J. **306:** 71-75, 1995.

GILLAN, M. G. C., KOSTERLITZ, H. W., AND PATERSON, S. J.: Comparison of the binding characteristics of tritiated opiates and opioid peptides. Br. J. Pharmacol. **70:** 481-490, 1980.

GILLAN, M. G. C., ROBSON, L. E., MCKNIGHT, A. T., AND KOSTERLITZ, H. W.: κ-binding and degradation of [^{3}H]dynorphin A (1-8) and [^{3}H]dynorphin A (1-9) in suspensions of guinea pig brain membranes. J. Neurochem. **45:** 1034-1042, 1985.

GINTZLER, A. R., AND XU, H.: Different G proteins mediate the opioid inhibition or enhancement of evoked [5-methionine]-enkephalin release. Proc. Natl. Acad. Sci. USA **88:** 4741-4745, 1991.

GIROS, B., POHL, M., ROCHELLE, J. M., AND SELDIN, M. F.: Chromosomal localization of opioid peptide and receptor genes in the mouse. Life Sci. **56:** PL369-PL375, 1995.

GOLDSTEIN, A., FISCHLI, W., LOWNEY, L. I., HUNKAPILLER, M., AND HOOD, L.: Porcine pituitary dynorphin: complete amino acid sequence of the biologically active heptadecapeptide. Proc. Natl. Acad. Sci. USA **78:** 7219-7223, 1981.

GOLDSTEIN, A., LOWNEY, L. I., AND PAL, B. K.: Stereospecific and nonspecific interactions of the morphine congener levorphanol in subcellular fractions of mouse brain. Proc. Natl. Acad. Sci. USA **68:** 1742-1747, 1971.

GOLDSTEIN, A., AND NAIDU, A.: Multiple opioid receptors: ligand selectivity profiles and binding site signatures. Mol. Pharmacol. **36:** 265-272, 1989.

GREVEL, J., YU, V., AND SADEE, W.: Characterization of a labile naloxone binding site (λ site) in rat brain. J. Neurochem. **44:** 1647-1656, 1985.

GRUDT, T. J., AND WILLIAMS, J. T.: κ-opioid receptors also increase potassium conductance. Proc. Natl. Acad. Sci. USA **90:** 11429-11432, 1993.

HADDAD, G. G., SCHAEFFER, J. I., AND CHANG, K. J.: Opposite effects of δ and μ-opioid receptor agonists on ventilation in conscious adult dogs. Brain. Res. **323:** 73-82, 1984.

HAHN, E. F., AND PASTERNAK, G. W.: Naloxonazine, a potent, long-lasting inhibitor of opiate binding sites. Life Sci. **31:** 1385-1388, 1982.

HAMON, M.: Mechanisms of opiate-induced immunodepression: current concepts. *In* Physiopathology of Illicit Drugs: Cannabis, Cocaine, Opiates. ed. by B. Nahas and C. Latour, Pergamon Press Adv. Biosci. series, vol. 80, pp. 277-283, 1991.

HANDA B. K., LAND, A. C., LORD, J. A., MORGAN, B. A., RANCE, M. J., AND SMITH, C. F.: Analogues of beta-LPH61-64 possessing selective agonist activity at mu-opiate receptors. Eur. J. Pharmacol. **70:** 531-540, 1981.

HANDLER, C. M., GELLER, E. B., AND ADLER, M. W.: Effect of μ-, κ-, and δ-selective opioid agonists on thermoregulation in the rat. Pharmacol. Biochem. Behav. **43:** 1209-1216, 1992.

HANDLER, C. M., PILIERO, T. C., GELLER, E. B., AND ADLER, M. W.: Effect of ambient temperature on the ability of mu-, kappa- and delta-selective opioid agonists to modulate thermoregulatory mechanisms in the rat. J. Pharmacol. Exp. Ther. **268:** 847-855, 1994.

HANSEN, P. E., AND MORGAN, B. A.: Structure-activity relationship in enkephalin peptides. *In* The Peptides, ed. by J. Meienhofer and S. Udenfriend, vol. 6, pp. 269-321, Academic Press, New York, 1984.

HASSEN, A. H., FEUERSTEIN, G., AND FADEN, A. I.: Kappa opioid receptors modulate cardiorespiratory function in hindbrain nuclei of rat. J. Neurosci. **4:** 2213-2221, 1984a.

HASSEN, A. H., FEUERSTEIN, G., AND FADEN, A. I.: Selective cadiorespiratory effects mediated by mu opioid receptors in the nucleus ambiguus. Neuropharmacology **23:** 407-415, 1984b.

HAWKINS, K. N., KNAPP, R. J., GEHLERT, D. R., LUI, G. K., YAMAMURA, M. S., ROESKE, L. C., HRUBY, V. J., AND YAMAMURA, H. I.: Quantitative autoradiography of [^{3}H] CTOP binding to mu opioid receptors in rat brain. Life Sci. **42:** 2541-2551, 1988.

HAWKINS, K. N., KNAPP, R. J., LUI, G. K., GULYA, K., KAZMIERSKI, W., WAN, Y. P., PELTON, J. T., HRUBY, V. J., AND YAMAMURA, H. I.: [^{3}H] - [H-D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH$_2$], ([^{3}H]CTOP), a potent and highly selective peptide for mu opioid receptors in rat brain. J. Pharmacol. Exp. Ther. **248:** 73-80, 1989.

HAYES, A. G., BIRCH, P. J., HAYWARD, N. J., SHEEHAN, M. J., ROGERS, H., TYERS, M. B., JUDD, D. B., SCOPES, D. I. C., AND NAYLOR, A.: A series of novel, highly potent and selective agonists for the κ-opioid receptor. Br. J. Pharmacol. **101:** 944-948, 1990.

HEYMAN, J. S, VAUGHT, J. L., RAFFA, R. B., AND PORRECA, F.: Can supraspinal delta-opioid receptors mediate antinociception? Trends Pharmacol. Sci. **9:** 134-138, 1988.

HILLER, J. M., ZHANG, Y., BING, G., GIOANNINI, T. L., STONE, E. A., AND SIMON, E. J.: Immunohistochemical localization of mu-opioid receptors in rat brain using antibodies generated against a peptide sequence present in a purified mu-opioid binding protein. Neuroscience **62:** 829-841, 1994.

HJORTH, S. A., THIRSTRUP, K., GRANDY, D. K., AND SCHWARTZ, T. W.: Analysis of selective binding epitopes for the κ-opioid receptor antagonist nor-binaltorphimine. Mol. Pharmacol. **47:** 1089-1094, 1995.

HOM, J. S. H., PAN, Y. X., BROOKS, A. I., STANDIFER, K. M., MATHIS, J. P., SCHEINBERG, D. A., AND PASTERNAK, G. W.: Characterization of the Kappa$_3$-like opioid receptor on Raji B lymphoma cells (Abstract). Soc. Neurosci. **21:** 527, 1995.

HONDA, C. N., AND ARVIDSSON, U.: Immunohistochemical localization of delta- and mu-opioid receptors in primate spinal cord. Neuroreport **6:** 1025-1028, 1995.

HORAN, P., DE COSTA, B. R., RICE, K. C., AND PORRECA, F.: Differential antagonism of U69,593- and bremazocine by (-)-UPHIT: evidence of kappa opioid receptor multiplicity in mice. J. Pharmacol. Exp. Ther. **257:** 1154-1161, 1991.

HORAN, P., TAYLOR, J., YAMAMURA, H. I., AND PORRECA, F.: Extremely long-lasting antagonistic actions of nor-binaltorphimine (nor-BNI) in the mouse tail-flick test. J. Pharmacol. Exp. Ther. **260:** 1237-1243, 1992.

HORAN, P. J., DE COSTA, B. R., RICE, K., HAASETH, R. C., HRUBY, V. J., AND PORRECA, F.: Differential antagonism of bremazocine- and U69,593-induced antinociception by quadazocine: further functional evidence of opioid κ receptor multiplicity in the mouse. J. Pharmacol. Exp. Ther. **266:** 926-933, 1993.

HUGHES, J., SMITH, T. W., KOSTERLITZ, H. W., FORTHERGILL, L. A., MORGAN, B. A., AND MORRIS, H. R.: Identification of two related pentapeptides from the brain with potent opiate agonist activity. Nature (Lond.) **258:** 577-579, 1975.

HUNTER, J. C., LEIGHTON, G. E., MEECHAM, K. G., BOYLE, S. J., HORWELL, D. C., REES, D. C., AND HUGHES, J.: CI-977, a novel and selective agonist for the κ-opioid receptor. Br. J. Pharmacol. **101:** 183-189, 1990.

HUTCHISON, M., KOSTERLITZ, H. W., LESIE, F. M., WATERFIELD, A. A., AND TERENIUS, L.: Assessment in the guinea pig ileum and mouse vas deferens of benzomorphans, which have antinociceptive activity but do not substitute for morphine in dependent monkey. Br. J. Pharmacol. **55:** 541-546, 1975.

IKEDA, K., KOBAYASHI, T., ICHIKAWA, T., USUI, H., AND KUMANISHI, T.: Functional coupling of the δ- and the κ-opioid receptors with the G-protein-activated K$^+$ channel. Biochem. Biophys. Res. Commun. **206:** 302-308, 1995.

IMPROTA, G., AND BROCCARDO, M.: Spinal antinociceptive effects of [D-Ala^2]deltorphin II, a novel and highly selective delta-opioid receptor agonist. Peptides **13:** 1123-1126, 1992.

ITZHAK, Y.: Multiple opioid binding sites. *In* The Opiate Receptors, ed. by G. W. Pasternak, pp. 95-142, Humana Press, Clifton, NJ, 1988.

JAMES, I. F., AND GOLDSTEIN, A.: Site-directed alkylation of multiple opioid receptors. I. Binding selectivity. Mol. Pharmacol. **25:** 337-342, 1984.

JIANG, Q., MOSBERG, H. L., AND PORRECA, F.: Antinociceptive effects of [D-Ala^2] deltorphin II, a highly selective δ agonist in vivo. Life Sci. **47:** PL43-PL47, 1990.

JIANG, Q., SEBASTIAN, A., ARCHER, S., AND BIDLACK, J. M.: 5β-methyl-14β-(p-nitrocinnamoylamino)-7,8-dihydromorphinone and its corresponding N-cyclopropylmethyl analog, N-cyclopropylmethylnor-5β-methyl-14β-(p-nitrocinnamoylamino)-7,8-dihydromorphinone: mu-selective irreversible opioid antagonists. J. Pharmacol. Exp. Ther. **268:** 1107-1113, 1994.

JIANG, Q., TAKEMORI, A. E., SULTANA, M., PORTOGHESE, P. S., BOWEN, W. D., MOSBERG, H. I., AND PORRECA, F.: Differential antagonism of opioid delta-antinociception by [D-ala^2, leu^5, Cys^6]enkephalin and naltrindole 5′-isothiocyanate: evidence for delta-receptor subtypes. J. Pharmacol. Exp. Ther. **257:** 1069-1075, 1991.

JIN, W., LEE, N. M., LOH, H. H., AND THAYER, S. A.: Opioids mobilize calcium from inositol 1,4,5-trisphosphate-sensitive stores in NG108-15 cells. J. Neurosci. **14:** 1920-1929, 1994.

JOHNSON, P. S., WANG, J. B., WANG, W. F., AND UHL, G. R.: Expressed mu opiate receptor couples to adenylate cyclase and phosphatidyl turnover. Neuroreport **5:** 507-509, 1994.

KAKIDANI, H., FURUTANI, Y., TAKAHASHI, H., NODA, M., MORIMOTO, Y., HIROSE, T., ASAI, M., INAYAMA, S., NAKANISHI, S., AND NUMA, S.: Cloning and sequence analysis of cDNA for porcine β-neo-endorphin/dynorphin precursor. Nature (Lond.) **298:** 245-249, 1982.

KANEKO, S., FUKUDA, K., YADA, N., AKAIKE, A., MORI, Y., AND SATOH, M.: Ca^{2+} channel inhibition by κ opioid receptors expressed in Xenopus oocytes. Neuroreport **5:** 2506-2508, 1994a.

KANEKO, S., NAKAMURA, S., ADACHI, K., AKAIKE, A., AND SATOH, M.: Mobilization of intracellular Ca^{2+} and stimulation of cyclic AMP production by κ opioid receptors in Xenopus oocytes. Mol. Brain Res. **27:** 258-264, 1994b.

KAUFMAN, D. L., XIA, J. R., KEITH, D. E., JR., NEWMAN, D., EVANS, C. J., AND LUSIS, A. J.: Localization of the delta opioid receptor to mouse chromosome 4 by linkage analysis. Genomics **19:** 405-406, 1994.

KAZMIERSKI, W., WIRE, W. S., LUI, G. K., KNAPP, R. J., SHOOK, J. E., BURKS, T. F., YAMAMURA, H. I., AND HRUBY, V. J.: Design and synthesis of somatostatin analogues with topographical properties that lead to highly potent and specific mu opioid receptor antagonists with greatly reduced binding at somatostatin receptors. J. Med. Chem. **31:** 2170-2177, 1988.

KEITH, D. E., JR., ANTON, B., AND EVANS, C. J.: Characterization and mapping of a delta opioid receptor clone from NG108-15 cells. Proc. West. Pharmacol. Soc. **36:** 299-306, 1993.

KIEFFER, B. L.: Recent advances in molecular recognition and signal transduction of active peptides: receptors for opioid peptides. Cell. Mol. Neurobiol. **15:** 615-635, 1995.

KIEFFER, B., BEFORT, K., GAVERIAUX-RUFF, C., AND HIRTH, C. G.: The delta opioid receptor: isolation of a cDNA by expression cloning and pharmacological characterization. Proc. Natl. Acad. Sci. USA **89:** 12048-12052, 1992.

KNAPP, R. J., MALATYNSKA, E., FANG, L., LI, X., BABIN, E., NGUYEN, M., SANTORO, G., VARGA, E. V., HRUBY, V. J., ROESKE, W. R., AND YAMAMURA, H.

I.: Identification of a human delta opioid receptor: cloning and expression. Life Sci. 54: PL463–PL469, 1994.

KNAPP, R. J., PORRECA, F., BURKS, T. F., AND YAMAMURA, H. I.: Mediation of analgesia by multiple opioid receptors. *In* Advances in Pain Research and Therapy, ed. by C. S. Hill Jr. and W. S. Fields, pp.247-289, Raven Press, New York, 1989.

KNAPP R. J., SHARMA, S. D., TOTH, G., DUONG, M. T., FANG, L., BOGERT, C. L., WEBER, S. J., HUNT, M., DAVIS, T. P., WAMSLEY, J. K., HRUBY, V. J., AND YAMAMURA, H. I.: [D-Pen^2, 4'-^{125}I-Phe^4, D-Pen^5]enkephalin: a selective high affinity radioligand for delta opioid receptors with exceptional specific activity. J. Pharmacol. Exp. Ther. 258: 1077-1083, 1991.

KONG, H., RAYNOR, K., YANO, H., TAKEDA, J., BELL, G., AND REISINE, T.: Agonists and antagonists bind to different domains of the cloned kappa opioid receptor. Proc. Natl. Acad. Sci. USA 91: 8042-8046, 1994.

KONG, H., RAYNOR, K., YASUDA, K., MOE, S. T., PORTOGHESE, P. S., BELL, G. I., AND REISINE, T.: A single residue, aspartic acid 95, in the delta opioid receptor specifies selective high affinity agonist binding. J. Biol. Chem. 268: 23055-23058, 1993.

KONKOY, C. S., AND CHILDERS, S. R.: Dynorphin-selective inhibition of adenylyl cyclase in guinea pig cerebellum membranes. Mol. Pharmacol. 36: 627-633, 1989.

KONKOY, C. S., AND CHILDERS, S. R.: Relationship between kappa 1 opioid receptor binding and inhibition of adenylate cyclase in guinea pig brain membranes. Biochem. Pharmacol. 45: 207-216, 1993.

KORLIPARA, V. L., TAKEMORI, A. E., AND PORTOGHESE, P. S.: N-benzylnaltrindoles as long-acting δ-opioid receptor antagonists. J. Med. Chem. 37: 1882-1885, 1994.

KOSTERLITZ, H. W., LORD, J. A. H., PATERSON, S. J., AND WATERFIELD, A. A.: Effects of changes in the structure of enkephalins and of narcotic analgesic drugs on their interactions with mu and delta receptors. Br. J. Pharmacol. 68: 333-342, 1980.

KOSTERLITZ, H. W., PATERSON, S. J., AND ROBSON, L. E.: Characterization of the κ-subtype of the opiate receptor in the guinea-pig brain. Br. J. Pharmacol. 73: 939-949, 1981.

KREIL, G., BARRA, D., SIMMACO, M., ERSPAMER, V., ERSPAMER, G. F., NEGRI, L., SEVERINI, C., CORSI, R., AND MELCHIORRI, P.: Deltorphin, a novel amphibian skin peptide with high selectivity and affinity for delta opioid receptors. Eur. J. Pharmacol. 163: 123-128, 1989.

KROMER, W.: The current status of opioid research on gastrointestinal motility. Life Sci. 44: 579-589, 1989.

KROMER, W.: Voltage-clamp experiments reveal receptor type-dependent modulation of chloride secretion in the guinea pig colonic mucosa by intestinal opioids. Naunyn-Schmiedebergs Arch. Pharmacol. 344: 360-367, 1991.

LAHTI, R. A., MICKELSON, M. M., MCCALL, J. M., AND VON VOIGTLANDER, P. F.: [^{3}H]U-69593, a highly selective ligand for the opioid κ receptor. Eur. J. Pharmacol. 109: 281-284, 1985.

LAHTI, R. A., VON VOIGTLANDER, P. F., AND BARSUHN, C.: Properties of a selective kappa agonist, U-50, 488 H. Life Sci. 31: 2257-2260, 1982.

LAI, H. W. L., MINAMI, M., SATOH, M., AND WONG, Y. H.: Gz coupling to the rat κ-opioid receptor. FEBS Lett. 360: 97-99, 1995.

LAI, J., MA, S. W., ZHU, R. H., ROTHMAN, R. B., LENTES, K. U., AND PORRECA, F.: Pharmacological characterization of the cloned kappa opioid receptor as a kappa1b subtype. Neuroreport 5: 2161-2164, 1994.

LAWRENCE, D. M. P., AND BIDLACK, J. M.: The kappa opioid receptor expressed on the mouse R1.1 thymoma cell line is coupled to adenylyl cyclase through a pertussis toxin-sensitive guanine nucleotide-binding regulatory protein. J. Pharmacol. Exp. Ther. 266: 1678-1683, 1993.

LAZARUS, L. H., BRYANT, S. D., ATTILA, M., AND SALVADORI, S.: Frog skin opioid peptides: a case for environmental mimicry. Environ. Health Perspect. 102: 648-654, 1994.

LAZARUS, L. H., WILSON, W. E., DE CASTIGLIONE, R. D., AND GUGLIETTA, A.: Dermorphin gene sequence peptide with high affinity and selectivity for δ-opioid receptors. J. Biol. Chem. 264: 3047-3050, 1989.

LEANDER, J. D.: A kappa opioid effect: increased urination in the rat. J. Pharmacol. Exp. Ther. 224: 89-94, 1983.

LEMAIRE, S., MAGNAN, J., AND REGOLI, D.: Rat vas deferens: a specific bioassay for endogenous opioid peptides. Br. J. Pharmacol. 64: 327-329, 1978.

LEVINE, A. S., GRACE, M., BILLINGTON, C. J., AND PORTOGHESE, P. S.: Norbinaltorphimine decreases deprivation and opioid-induced feeding. Brain Res. 534: 60-64, 1990.

LEYSEN, J. E., GOMMEREN, W., AND NIEMEGEERS, C. J. E.: [^{3}H]Sufentanyl, a superior ligand for μ-opiate receptors: binding properties and regional distribution in rat brain and spinal cord. Eur. J. Pharmacol. 87: 209-225, 1983.

LI, C. H., AND CHUNG, D.: Isolation and structure of an untriakontapeptide with opiate activity from camel pituitary glands. Proc. Natl. Acad. Sci. USA 73: 1145-1148, 1976.

LI, S., ZHU, J., CHEN, C., CHEN, Y. W., DERIEL, J. K., ASHBY, B., AND LIU-CHEN, L. Y.: Molecular cloning and expression of a rat κ opioid receptor. Biochem. J. 295: 629-633, 1993.

LIU-CHEN, L. Y., LI, S. X., AND LEWIS, M. E.: Autoradiographic study of irreversible binding of [^{3}H]beta-funaltrexamine to opioid receptors in the rat forebrain: comparison with mu and delta receptor distribution. Brain Res. 544: 235-242, 1991.

LOOSE, M. D., AND KELLY, M. J.: Opioids act at μ-receptors to hyperpolarize arcuate neurons via an inwardly rectifying potassium conductance. Brain Res. 513: 15-23, 1990.

LORD, J. A. H., WATERFIELD, A. A., HUGHES, J., AND KOSTERLITZ, H. W.: Endogenous opioid peptides: multiple agonists and receptors. Nature (Lond.) 267: 495-499, 1977.

LUNG, F. D. T., MEYER, J. P., LI, G., LOU, B. S., STROPOVA, D., DAVIS, P., YAMAMURA, H. I., PORRECA, F., AND HRUBY, V. J.: Highly κ receptor-selective dynorphin A analogues with modifications in position 3 of dynorphin A(1-11)-NH_2. J. Med. Chem. 38: 585-586, 1995.

MAGNAN, J., PATERSON S. J., TAVANI, A., AND KOSTERLITZ, H. W.: The binding spectrum of narcotic analgesic drugs with different agonist and antagonist properties. Naunyn-Schmiedebergs Arch. Pharmacol. 319: 197-205, 1982.

MAILMAN, D.: Morphine-neural interactions on canine intestinal absorption and blood flow. Br. J. Pharmacol. 81: 263-270, 1984.

MANNALACK, D. T., BEART, P. M., AND GUNDLACH, A. L.: Psychotomimetic σ-opiates and PCP. Trends Pharmacol. Sci. 7: 448-451, 1986.

MANSOUR, A., FOX, C. A., AKIL, H., AND WATSON, S. J.: Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. Trends Neurosci. 18: 22-29, 1995.

MANSOUR, A., FOX, C. A., BURKE, S., MENG, F., THOMPSON, R. C., AKIL, H., AND WATSON, S. J.: Mu, delta, and kappa opioid receptor mRNA expression in the rat CNS: an in situ hybridization study. J. Comp. Neurol. 350: 412-438, 1994.

MANSOUR, A., KHACHATURIAN, H., LEWIS, M. E., AKIL, H., AND WATSON, S. J.: Anatomy of CNS opioid receptors. Trends Neurosci. 11: 308-314, 1988.

MANSOUR, A., LEWIS, M. E., KHACHATURIAN, H., AKIL, H., AND WATSON, S. J.: Pharmacological and anatomical evidence of selective μ, δ and κ opioid receptor binding in rat brain. Brain Res. 398: 69-79, 1986.

MARKI, A., OTVOS, F., TOTH, G., HOSZTAFI, S., AND BORSODI, A.: Characterization of kappa opioid receptors with tritiated norBNI. Analgesia 1: 557-560, 1995.

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

MARTINKA, G. P., JHAMANDAS, K., SABOURIN, L., LAPIERRE, C., AND LEMAIRE, S.: Dynorphin A-(1-13)-Tyr^{14}-Leu^{15}-Phe^{16}-Asn^{17}-Gly^{18}-Pro^{19}: a potent and selective κ opioid peptide. Eur. J. Pharmacol. 196: 161-167, 1991.

MATHIASEN, J. R., AND VAUGHT, J. L.: [D-Pen^2, L-Pen^5] enkephalin induced analgesia in the jimpy mouse: in vivo evidence for δ-receptor mediated analgesia. Eur. J. Pharmacol. 136: 405-407, 1987.

MAURER, R., GAEHWILER, B. H., BUCHER, H. H., HILL, R. C., AND ROEMER, D.: Opiate antagonist properties of an octapeptide somatostatin analog. Proc. Natl. Acad. Sci. USA 79: 4815-4817, 1982.

MCKENZIE, F. R., AND MILLIGAN, G.: δ-opioid-receptor-mediated inhibition of adenylate cyclase is transduced specifically by the guanine-nucleotide-binding protein Gi2. Biochem. J. 267: 391-398, 1990.

MCKNIGHT, A. T., CORBETT, A. D., MARCOLI, M., AND KOSTERLITZ, H. W.: The opioid receptors in the hamster vas deferens are of the δ-type. Neuropharmacology 24: 1011-1018, 1985.

MCKNIGHT, A. T., AND REES, D. C.: Opioid receptors and their ligands. Neurotransmissions (Res. Biochem. Inc.) 7: 1-6, 1991.

MENG, F., XIE, G. X., THOMPSON, R., MANSOUR, A., GOLDSTEIN, A., WATSON, S., AND AKIL, H.: Cloning and pharmacological characterization of a rat kappa opioid receptor. Proc. Natl. Acad. Sci. USA 90: 9954-9958, 1993.

MEUNIER, J. C., MOLLEREAU, C., TOLL, L., SUAUDEAU, C., MOISAND, C., ALVINERIE, P., BUTOUR, J. L., GUILLEMOT, J. C., FERRARA, P., MONSARRAT, B., MAZARGUIL, H.., VASSART, G., PARMENTIER, M., AND COSTENTIN, J.: Isolation and structure of the endogenous agonist of opioid receptor-like ORL_1 receptor. Nature (Lond.) 377: 532-535, 1995.

MEYER, M. E., AND MEYER, M. E.: Behavioral effects of opioid peptide agonists DAMGO, DPDPE and DAKLI on locomotor activities. Pharmacol. Biochem. Behav. 45: 315-320, 1993.

MIHARA, S., AND NORTH, R. A.: Opioids increase potassium conductance in submucous neurones of guinea-pig caecum by activating delta-receptors. Br. J. Pharmacol. 88: 312-322, 1986.

MILLAN, M. J.: Kappa-opioid receptor-mediated antinociception in the rat. I. Comparative actions of mu- and kappa-opioids against noxious thermal, pressure and electrical stimuli. J. Pharmacol. Exp. Ther. 251: 334-341, 1989.

MILLAN, M. J., CZLONKOWSKI, A., LIPKOWSKI, A., AND HERZ, A.: Kappa-opioid receptor-mediated antinociception in the rat. II. Supraspinal in addition to spinal sites of action. J. Pharmacol. Exp. Ther. 251: 342-350, 1989.

MIN, B. H., AUGUSTIN, L. B., FELSHEIM, R. F., FUCHS, J. A., AND LOH, H. H.: Genomic structure and analysis of promoter sequence of a mouse μ opioid receptor gene. Proc. Natl. Acad. Sci. USA 91: 9081-9085, 1994.

MINAMI, M., ONOGI, T., NAKAGAWA, T., KATAO, Y., AOKI, Y., KATSUMATA, S., AND SATOH, M.: DAMGO, a mu-opioid receptor selective ligand, distinguishes mu- and kappa-opioid receptors at a different region from that for the distinction between mu- and delta-opioid receptors. FEBS Lett. 364: 23-27, 1995.

MINAMI, M., ONOGI, T., TOYA, T., KATAO, Y., HOSOI, Y., MAEKAWA, K., KATSUMATA, S., YABUUCHI, K., AND SATOH, M.: Molecular cloning and in situ hybridization histochemistry for rat mu-opioid receptor. Neurosci. Res. 18: 315-322, 1994.

MINAMI, M., TOYA, T., KATAO, Y., MAEKAWA, K., NAKAMURA, S., ONOGI, T., KANEKO, S., AND SATOH, M.: Cloning and expression of a cDNA for the rat kappa-opioid receptor. FEBS Lett. 329: 291-295, 1993.

MISAWA, H., UEDA, H., KATADA, T., UI, M., AND SATOH, M.: A subtype of opioid kappa-receptor is coupled to inhibition of Gi1-mediated phospholipase C activity in the guinea pig cerebellum. FEBS Lett. 361: 106-110, 1995.

MISAWA, H., UEDA, H., AND SATOH, M.: κ-opioid agonist inhibits phospholipase C, possibly via an inhibition of G-protein activity. Neurosci. Lett. 112: 324-327, 1990.

MOISES, H. C., RUSIN, K. I., AND MACDONALD, R. L.: μ-opioid receptor-mediated reduction of neuronal calcium current occurs via a Go-type GTP-binding protein. J. Neurosci. 14: 3642-3651, 1994a.

MOISES, H. C., RUSIN, K. I., AND MACDONALD, R. L.: μ- and κ-opioid receptors selectively reduce the same transient components of high-threshold calcium current in rat dorsal root ganglion sensory neurons. J. Neurosci. 14: 5903-5916, 1994b.

MOLLEREAU, C., PARMENTIER, M., MAILLEUX, P., BUTOUR, J. L., MOISAND, C., CHALON, P., CAPUT, P., VASSART, G., AND MEUNIER, J. C.: ORL1, a novel member of the opioid receptor family. Cloning, functional expression and localization. FEBS Lett. 341: 33-38, 1994.

MOOLTEN, M. S., FISHMAN, J. B., CHEN, J. C., AND CARLSON, K. R.: Etonitazene: an opioid selective for the mu receptor types. Life Sci. 52: PL199–PL203, 1993.

MORIN-SURUN, M. P., BOUDINOT, E., GACEL, G., CHAMPAGNAT, J., ROQUES, B. P., AND DENAVIT-SAUBIÉ, M.: Different effects of μ and δ opiate agonists on respiration. Eur. J. Pharmacol. 98: 235-240, 1984.

MOSBERG, H. I., HURST, R., HRUBY, V. J., GEE, K., YAMAMURA, H. I., GALLIGAN, J. J., AND BURKS, T. F.: Bis-penicillinamine enkephalins possess highly improved specificity toward δ opioid receptors. Proc. Natl. Acad. Sci. USA 80: 5871-5874, 1983.

MOYSE, E., PASQUINI, F., QUIRION, R., AND BEAUDET, A.: ^{125}I-FK 33-824: a selective probe for autoradiographic labeling of μ opioid receptors in the brain. Peptides 7: 351-355, 1986.

MUCHA, R. F., AND HERZ, A.: Motivational properties of kappa and mu opioid receptor agonists studied with place and taste preference conditioning. Psychopharmacology 86: 274-280, 1985.

NAKANISHI, S., INOUE, A., KITA, T., NAKAMURA, M., CHANG, A. C. Y., COHEN, S. N., AND NUMA, S.: Nucleotide sequence of cloned cDNA for bovine corticotropin-beta-lipotropin precursor. Nature (Lond.) 278: 423-427, 1979.

NAKAZAWA, T., FURUYA, Y., KANEKO, T., AND YAMATSU, K.: Spinal kappa receptor-mediated analgesia of E-2078, a systemically active dynorphin analog, in mice. J. Pharmacol. Exp. Ther. 256: 76-81, 1991.

NEGRI, L., FALCONIERI ERSPAMER, G., SEVERINI, C., POTENZA, R. L., MELCHIORRI, P., AND ERSPAMER, V.: Dermorphin-related peptides from the skin of Phyllomedusa bicolor and their amidated analogs activate two μ opioid receptor subtypes that modulate antinociception and catalepsy in the rat. Proc. Natl. Acad. Sci. USA 89: 7203-7207, 1992.

NEVIN, S. T., TOTH, G., NGUYEN, T. M. D., SCHILLER, P. W., AND BORSODI, A.: Synthesis and binding characteristics of the highly specific tritiated delta opioid antagonist [^{3}H] TIPP. Life Sci. 53: PL57–PL62, 1993.

NEVIN, S. T., TOTH, G., WELTROWSKA, G., SCHILLER, P. W., AND BORSODI, A.: Synthesis and binding characteristics of tritiated TIPP[Ψ], a highly specific and stable delta opioid antagonist. Life Sci. 56: PL225–PL230, 1995.

NIJSSEN, P. C. G., SEXTON, T., AND CHILDERS, S. R.: Opioid-inhibited adenylyl cyclase in rat brain membranes: lack of correlation with high-affinity opioid receptor binding sites. J. Neurochem. 59: 2251-2262, 1992.

NISHI, M., TAKESHIMA, H., FUKUDA, K., KATO S., AND MORI, K.: cDNA cloning and pharmacological characterization of an opioid receptor with high affinities for kappa-subtype selective ligands. FEBS Lett. 330: 77-80, 1993.

NOBLE F., AND COX, B. M.: Differential regulation of D1 dopamine receptor- and of A2a adenosine receptor-stimulated adenylate cyclase by μ-, δ1-, and δ2-opioid agonists in rat caudate putamen. J. Neurochem. 65: 125-133, 1995.

NOCK, B., GIORDANO, A. L., AND CICERO, T. J.: ICI 197067 is a selective ligand for the U-69593-sensitive kappa opiate binding site. Eur. J. Pharmacol. 162: 385-386, 1989.

NOCK, B., GIORDANO, A. L., CICERO, T. J., AND O'CONNOR, L. H.: Affinity of drugs and peptides for U-69,593-sensitive and -insensitive kappa opiate binding sites: the U-69,593-insensitive site appears to be the beta endorphin-specific epsilon receptor. J. Pharmacol. Exp. Ther. 254: 412-419, 1990.

NOCK, B., GIORDANO, A. L., MOORE, B. W., AND CICERO, T. J.: Properties of the putative epsilon receptor—identification in rat, guinea-pig, cow, pig and chicken brain. J. Pharmacol. Exp. Ther. 264: 349-359, 1993.

NOCK, B., RAJPARA, A., O'CONNOR, L. H., AND CICERO, T. J.: Autoradiography of [^{3}H]U-69593 binding sites in rat brain: evidence for κ opioid receptor subtypes. Eur. J. Pharmacol. 154: 27-34, 1988.

NODA, M., FURUTANI, Y., TAKAHASHI, H., TOYOSATO, M., HIROSE, T., INAYAMA, S., NAKANISHI, S., AND NUMA, S.: Cloning and sequence analysis of cDNA for bovine adrenal preproenkephalin. Nature (Lond.) 295: 202-206, 1982.

OLIANAS, M. C., AND ONALI, P.: Characterization of opioid receptors mediating stimulation of adenylate cyclase activity in rat olfactory bulb. Mol. Pharmacol. 42: 109-115, 1992.

OLIANAS, M. C., AND ONALI, P.: Activation of opioid and muscarinic receptors stimulates basal adenylyl cyclase but inhibits Ca^{2+}/calmodulin- and forskolin-stimulated enzyme activities in rat olfactory bulb. J. Neurochem. 63: 161-168, 1994.

OLMSTED, S. L., TAKEMORI, A. E., AND PORTOGHESE, P. S.: A remarkable change of opioid receptor selectivity on the attachment of a peptidomimetic kappa address element to the delta antagonist, naltrindole: 5′-[N^2-(alkylamidino)methyl] naltrindole derivatives as a novel class of kappa opioid receptor antagonists. J. Med. Chem. 36: 179-180, 1993.

ONALI, P., AND OLIANAS, M. C.: Naturally occurring opioid receptor agonists stimulate adenylate cyclase activity in rat olfactory bulb. Mol. Pharmacol. 39: 436-441, 1991.

ONOGI, T., MINAMI, M., KATAO, Y., NAKAGAWA, T., AOKI, Y., TOYA, T., KATSUMATA, S., AND SATOH, M.: DAMGO, a mu-opioid receptor selective agonist, distinguishes mu- and delta-opioid receptors around their first extracellular loop. FEBS Lett. 357: 93-97, 1995.

PASTERNAK, G. W., SIMANTOV, R., AND SNYDER S. H.: Characterization of an endogenous morphine-like factor (enkephalin) in mammalian brain. Mol. Pharmacol. 12: 504-513, 1976.

PASTERNAK, G. W., AND STANDIFER, K. M.: Mapping of opioid receptors using antisense oligodeoxynucleotides: correlating their molecular biology and pharmacology. Trends Pharmacol. Sci. 16: 344-350, 1995.

PASTERNAK, G. W., AND WOOD, P. J.: Multiple mu opiate receptors. Life Sci. 38: 1889-1898, 1986.

PAUL, D., BODNAR, R. J., GISTRAK, M. A., AND PASTERNAK, G. W.: Different receptor subtypes mediate spinal and supraspinal analgesia in mice. Eur. J. Pharmacol. 168: 307-314, 1989.

PAUL, D., LEVISON, J. A., HOWARD, D. H., PICK, C. G., HAHN, E. F., AND PASTERNAK, G. W.: Naloxone benzoylhydrazone (NalBzOH) analgesia. J. Pharmacol. Exp. Ther. 255: 769-774, 1990.

PAZOS, A., AND FLOREZ, J.: A comparative study in rats of the respiratory depression and analgesia induced by μ- and δ-opioid agonists. Eur. J. Pharmacol. 99: 15-21, 1984.

PELTON, J. T., GULYA, K., HRUBY, V. J., DUCKLES, S. P., AND YAMAMURA, H. I.: Conformationally restricted analogs of somatostatin with high mu-opiate receptor specificity. Proc. Natl. Acad. Sci. USA 82: 236-239, 1985.

PELTON, J. T., KZAMIERSKI, W., GULYA, K., YAMAMURA, H. I., AND HRUBY, V. J.: Design and synthesis of conformationally constrained somatostatin analogues with high potency and specificity for μ opioid receptors. J. Med. Chem. 29: 2370-2375, 1986.

PERT, C. B., AND SNYDER, S. H.: Opiate receptor: demonstration in nervous tissue. Science (Wash. DC) 179: 1011-1014, 1973.

PFEIFFER, A., BRANTL, V., HERZ, A., AND EMRICH, H. M.: Psychotomimesis mediated by κ opiate receptors. Science (Wash. DC) 233: 774-776, 1986.

PICK, C. G., PAUL, D., AND PASTERNAK, G. W.: Nalbuphine, a mixed kappa1 and kappa3 analgesic in mice. J. Pharmacol. Exp. Ther. 263: 1044-1050, 1992.

POL, O., FERRER, I., AND PUIG, M. M.: Diarrhea associated with intestinal inflammation increases the potency of mu and delta opioids on the inhibition of gastrointestinal transit in mice. J. Pharmacol. Exp. Ther. 270: 386-391, 1994.

POLASTRON, J., BOYER, M. J., QUERTERMONT, Y., THOUVENOT, J. P., MEUNIER, J. C., AND JAUZAC, P.: μ-opioid receptors and not κ-opioid receptors are coupled to the adenylate cyclase in the cerebellum. J. Neurochem. 54: 562-570, 1990.

PORRECA, F., MOSBERG, H. I., HURST, R., HRUBY, V. J., AND BURKS, T. F.: Roles of mu, delta and kappa opioid receptors in spinal and supraspinal mediation of gastrointestinal transit effects and hot-plate analgesia in the mouse. J. Pharmacol. Exp. Ther. 230: 341-348, 1984.

PORRECA, F., MOSBERG, H. I., OMNAAS, J. R., BURKS, T. F., AND COWAN, A.: Supraspinal and spinal potency of selective opioid agonists in the mouse writhing test. J. Pharmacol. Exp. Ther. 240: 890-894, 1987.

PORTOGHESE, P. S.: A new concept on the mode of interaction of narcotic analgesics with receptors. J. Med. Chem. 8: 609-616, 1965.

PORTOGHESE, P. S., FAROUZ-GRANT, F., SULTANA, M., AND TAKEMORI, A. E.: 7′-Substituted amino acid conjugates of naltrindole. Hydrophilic groups as determinants of selective antagonism of δ1 opioid receptor-mediated antinociception in mice. J. Med. Chem. 38: 402-407, 1995.

PORTOGHESE, P. S., LARSON, D. L., SAYRE, L. M., FRIES, D. S., AND TAKEMORI, A. E.: A novel opioid receptor site directed alkylating agent with irreversible narcotic antagonistic and reversible agonistic activities. J. Med. Chem. 23: 233-234, 1980.

PORTOGHESE, P. S., LIPKOWKSI, A. W., AND TAKEMORI, A. E.: Bimorphinans as highly selective potent kappa receptor antagonists. J. Med. Chem. 30: 238-239, 1987.

PORTOGHESE, P. S., MOE, S. T., AND TAKEMORI, A. E.: A selective δ1 opioid receptor agonist derived from oxymorphone. Evidence for separate recognition sites for δ1 opioid receptor agonists and antagonists. J. Med. Chem. 36: 2572-2574, 1993.

PORTOGHESE, P. S., AND TAKEMORI, A. E.: TENA, a selective kappa opioid receptor antagonist. Life Sci. 36: 801-805, 1985.

PORTOGHESE, P. S., SULTANA, M., NAGASE, H., AND TAKEMORI, A. E.: A highly selective δ1-opioid receptor antagonist: 7-benzylidenenaltrexone. Eur. J. Pharmacol. 218: 195-196, 1992a.

PORTOGHESE, P. S., SULTANA, M., NELSON, W. L., KLEIN, P., AND TAKEMORI, A. E.: δ opioid antagonist activity and binding studies of regioisomeric isothiocyanate derivatives of naltrindole: evidence for δ receptor subtypes. J. Med. Chem. 35: 4086-4091, 1992b.

PORTOGHESE, P. S., SULTANA, M., AND TAKEMORI, A. E.: Naltrindole, a highly

selective and potent non-peptide δ opioid receptor antagonist. Eur. J. Pharmacol. **146:** 185-186, 1988.

PRATHER, P. L., LOH, H. H., AND LAW, P. Y.: Interaction of δ-opioid receptors with multiple G proteins: a non-relationship between agonist potency to inhibit adenylyl cyclase and to activate G proteins. Mol. Pharmacol. **45:** 997-1003, 1994.

PRATHER, P. L., MCGINN, T. M., CLAUDE, P. A., LIUCHEN, L. Y., LOH, H. H., AND LAW, P. Y.: Properties of a kappa-opioid receptor expressed in CHO cells: interaction with multiple G-proteins is not specific for any individual G alpha subunit and is similar to that of other opioid receptors. Mol. Brain Res. **29:** 336-346, 1995.

PRICE, M., GISTRAK, M. A., ITZHAK, Y., HAHN, E. F., AND PASTERNAK, G. W.: Receptor binding of ^{3}H-naloxone benzoylhydrazone: a reversible κ and slowly dissociable mu opiate. Mol. Pharmacol. **35:** 67-74, 1989.

PRIMI, M. P., FARGEAS, M. J., AND BUENO, L.: Central μ-, δ-, and κ-opioid influences on intestinal water and electrolyte transport in dogs. Regul. Pept. **21:** 107-115, 1988.

RAGHUBIR, R., PATNAIK, G. K., SHARMA, S. D., MATHUR, K. B., AND DHAWAN, B. N.: Pharmacological profile of two new analogues of metenkephalin. *In* Recent Progress in the Chemistry and Biology of Centrally Acting Peptides, ed. by B. N. Dhawan and R. S. Rapaka, pp. 167-174, Central Drug Research Institute, Lucknow, India, 1988.

RAGHUBIR, R., SRIMAL, R. C., AND DHAWAN, B. N.: Enkephalinergic modulation of cardiovascular effects of clonidine from ventral surface of medulla in cat. *In* Brain Neurotransmitter Mechanism and Hypertension, ed. by K. K. Tangri, S. Vrat, and A. K. Saxena, pp. 109-115, Kamla Printers, Lucknow, India, 1987.

RANDICH, A., ROBERTSON, J. D., AND WILLINGHAM, T.: The use of specific opioid agonists and antagonists to delineate the vagally mediated antinociceptive and cardiovascular effects of intravenous morphine. Brain Res. **603:** 186-200, 1993.

RAYNOR, K., KONG, H., CHEN, Y., YASUDA, K., YU, L., BELL, G., AND REISINE, T.: Pharmacological characterization of cloned kappa, delta and mu opioid receptors. Mol. Pharmacol. **45:** 330-334, 1994.

RAYNOR, K., KONG, H., MESTEK, A., BYE, L. S., TIAN, M., YU, J., AND REISINE, T.: Characterization of the cloned human mu opioid receptor. J. Pharmacol. Exp. Ther. **272:** 423-428, 1995.

REINSCHEID, R. K., NOTHACKER, H. P., BOURSON, A., ARDATI, A., HENNINGSEN, R. A., BUNZOW, J. R., GRANDY, D. K., LANGEN, H., MONSMA, F. J., JR., AND CIVELLI, O.: Orphanin FQ: a neuropeptide that activates an opioid like G protein-coupled receptor. Science (Wash. DC) **270:** 792-794, 1995.

REISINE, T., AND BELL, G. I.: Molecular biology of opioid receptors. Trends Neurosci. **16:** 506-510, 1993.

RENDA, T., NEGRI, L., TOOYAMA, I., CASU, C., AND MELCHIORRI, P.: Autoradiographic study on [^{3}H]-[D-Ala^2]-deltorphin-I binding sites in the rat brain. Neuroreport **4:** 1143-1146, 1993.

RHIM, H., AND MILLER, R. J.: Opioid receptors modulate diverse types of calcium channels in the nucleus tractus solitarius of the rat. J. Neurosci. **14:** 7608-7615, 1994.

RICHARDSON, A., DEMOLIOU-MASON, C., AND BARNARD, E. A.: Guanine nucleotide-binding protein-coupled and uncoupled states of opioid receptors and their relevance to the determination of subtypes. Proc. Natl. Acad. Sci. USA **89:** 10198-10202, 1992.

RICHTER, K., EGGER, R., NEARI, L., CORSI, R., SEVRINI, C., AND KREIL, G.: cDNA encoding [D-ala^2] deltorphin precursors from skin of Phyllomedusa bicolor also contain genetic information for three dermorphin - related opioid peptides. Proc. Natl. Acad. Sci. USA **87:** 4836-4839, 1990.

ROEMER, D., BUSCHER, H. H., HILL, R. C., PLESS, J., BAUER, W., CARDINAUX, F., CLOSSE, A., HAUSER, D., AND HUGUENIN, R.: A synthetic enkephalin analog with prolonged parenteral and oral analgesic activity. Nature (Lond.) **268:** 547-549, 1977.

ROERIG, S. C., LOH, H. H., AND LAW, P. Y.: Identification of three separate guanine nucleotide-binding proteins that interact with the δ-opioid receptor in NG108–15 neuroblastoma × glioma hybrid cells. Mol. Pharmacol. **41:** 822-831, 1992.

ROGERS H., BIRCH, P. J., HARRISON, S. M., PALMER, E., MANCHEE, G. R., JUDD, D. B., NAYLOR, A., SCOPES, D. I. C., AND HAYES, A. G.: GR 94839, a κ-opioid agonist with limited access to the central nervous system, has antinociceptive activity. Br. J. Pharmacol. **106:** 783-789, 1992.

RÖMER, D., BÜSCHER, H., HILL, R. C., MAURER, R., PETCHER, T. J., WELLE, H. B. A., BAKEL, C. C. K., AND AKKERMAN, A. M.: Bremazocine: a potent, long-acting opiate kappa-agonist. Life Sci. **27:** 971-978, 1980.

RÖMER, D., BÜSCHER, H. H., HILL, R. C., MAURER, R., PETCHER, T. J., ZEUGNER, H., BENSON, W., FINNER, E., MILKOWSKI, W., AND THIES, P. W.: An opioid benzodiazepine. Nature (Lond.) **298:** 759-760, 1982.

ROSSI, G. C., PAN, Y. X., BROWN, G. P., AND PASTERNAK, G. W.: Antisense mapping the MOR-1 opioid receptor: evidence for alternative splicing and a novel morphine-6 beta-glucuronide receptor. FEBS Lett. **369:** 192-196, 1995.

ROTHMAN, R. B., BYKOV, V., XUE, B. G., XU, H., DE COSTA, B. R., JACOBSON, A. E., RICE, K. C., KLEINMAN, J. E., AND BRADY, L. S.: Interaction of opioid peptides and other drugs with multiple kappa receptors in rat and human brain: evidence for species differences. Peptides **13:** 977-987, 1992.

SATOH, M., AND MINAMI, M.: Molecular pharmacology of the opioid receptors. Pharmacol. Ther. **68:** 343-364, 1995.

SCHILLER, P. W., NGUYEN, T. M. D., WELTROWSKA, G., WILKES, B. C., MARSDEN, B. J., LEMIEUX, C., AND CHUNG, N. N.: Differential stereochemical requirements of μ vs δ opioid receptors for ligand binding and signal transduction: development of a class of potent and highly δ-selective peptide antagonists. Proc. Natl. Acad. Sci. USA **89:** 11871-11875, 1992.

SCHILLER, P. W., WELTROWSKA, G. NGUYEN, T. M. D., WILLKES, V. C., CHUNG, N. N., AND LEMI, E. U. X.: TIPP [Ψ]: a highly potent and stable pseudopeptide delta opioid receptor antagonist with extraordinary delta selectivity. J. Med. Chem. **36:** 3182-3187, 1993.

SCHMAUSS, C.: Spinal κ-opioid receptor-mediated antinociception is stimulus specific. Eur. J. Pharmacol. **137:** 197-205, 1987.

SCHMAUSS, C., AND YAKSH, T. L.: In vivo studies on spinal opiate receptor systems mediating antinociception. II. Pharmacological profiles suggesting a differential association of mu, delta and kappa receptors with visceral and cutaneous thermal stimuli in the rat. J. Pharmacol. Exp. Ther. **228:** 1-11, 1984.

SCHOFIELD, P. R., MCFARLAND, K. C., HAYFLICK, J. S., WILCOX, J. N., CHO, T. M., ROY, S., LEE, N. M., LOH, H. H., AND SEEBURG, P. H.: Molecular characterization of a new immunoglobulin superfamily protein with potential roles in opioid binding and cell contact. EMBO J. **8:** 489-495, 1989.

SCHULZ, R., WUSTER, M., AND HERZ, A.: Pharmacological characterization of the ε opiate receptor. J. Pharmacol. Exp. Ther. **216:** 604-606, 1981.

SHAW, J. S., MILLER, L. TURNBULL, M. J., GORMLEY, J. J., AND MORLEY, J. S.: Selective antagonist at the opiate delta-receptors. Life Sci. **31:** 1259-1262, 1982.

SHEN, K. F., AND CRAIN, S. M.: Cholera toxin-A subunit blocks opioid excitatory effects on sensory neuron action potential indicating mediation by Gs linked opioid receptors. Brain Res. **525:** 225-231, 1990.

SHOOK, J. E., KAZMIERSKI, W, WIRE W. S., LEMCKE, P. K., HRUBY, V. J., AND BURKS, T. F.: Opioid receptor selectivity of β-endorphin in vitro and in vivo: mu, delta and epsilon receptors. J. Pharmacol. Exp. Ther. **246:** 1018-1025, 1988.

SIMON, E. J., HILLER, J. M., AND EDELMAN, I.: Stereospecific binding of the potent narcotic analgesic [^{3}H] etorphine to rat brain homogenate. Proc. Natl. Acad. Sci. USA **70:** 1947-1949, 1973.

SOFUOGLU, M., PORTOGHESE, P. S., AND TAKEMORI, A. E.: 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, 1991.

SPANAGEL, R., ALMEIDA, O. F. X., AND SHIPPENGERG, T. S.: Evidence that nor-binaltorphimine can function as an antagonist at multiple opioid receptor subtypes. Eur. J. Pharmacol. **264:** 157-162, 1994.

SPIEGEL, K., AND PASTERNAK, G. W.: Meptazinol: a novel mu-1 selective analgesic. J. Pharmacol. Exp. Ther. **228:** 414-419, 1984.

SRIMAL, R. C., RAGHUBIR, R., AND DHAWAN, B. N.: Cardiovascular response to enkephalin on topical application to ventral surface of medulla. *In* Current Status of Centrally Acting Peptides, ed. by B. N. Dhawan, pp.77-83, Pergamon Press, Oxford, 1982.

STAPELFELD, A., HAMMOND, D. L., AND RAFFERTY, M. F.: Antinociception after intra-cerebroventricular administration of naltrindole in the mouse. Eur. J. Pharmacol. **214:** 273-276, 1992.

STEIN, C.: Peripheral mechanisms of opioid analgesia. Anesth. Analg. **76:** 182-191, 1993.

STEWART P. E., AND HAMMOND, D. L.: Evidence for delta opioid receptor subtypes in rat spinal cord: studies with intrathecal naltriben, cyclic[D-Pen^2, D-Pen^5] enkephalin and [D-Ala^2, Glu^4] deltorphin. J. Pharmacol. Exp. Ther. **266:** 820-828, 1993.

SULLIVAN, A. F., DICKENSON, A. H., AND ROQUES, B. P.: δ-opioid mediated inhibitions of acute and prolonged noxious-evoked responses in rat dorsal horn neurones. Br. J. Pharmacol. **98:** 1039-1049, 1989.

SURRATT, C. K., JOHNSON, P. S., MORIWAKI, A., SEIDLECK, B. K., BLASCHAK, C. J., WANG, J. B., AND UHL, G. R.: μ Opiate receptor. Charged transmembrane domain amino acids are critical for agonist recognition and intrinsic activity. J. Biol. Chem. **269:** 20548-20553, 1994.

TAKEMORI, A. E., SULTANA, M., NAGASE, H., AND PORTOGHESE, P. S.: Agonist and antagonist activities of ligands derived from naltrexone and oxymorphone. Life Sci. **50:** 1491-1495, 1992.

TALLENT, M., DICHTER, M. A., BELL, G. I., AND REISINE, T.: The cloned kappa opioid receptor couples to an N-type calcium current in undifferentiated PC-12 cells. Neuroscience **63:** 1033-1040, 1994.

TAMIR, M., AND KUSHNER, L.: Expression of functional δ opioid receptor in Xenopus oocytes. Biochem. Biophys. Res. Commun. **193:** 1224-1231, 1993.

TAUSSIG, R., SANCHEZ, S., RIFO, M., GOLMAN, A. G., AND BELARDETTI, F.: Inhibition of the omega conotoxin-sensitive calcium current by distinct G proteins. Neuron **8:** 799-809, 1992.

TEMPEL, A., AND ZUKIN, R. S.: Neuroanatomical patterns of the μ, δ and κ opioid receptors of rat brain as determined by quantitative in vitro autoradiography. Proc. Natl. Acad. Sci. USA **84:** 4308-4312, 1987.

TERENIUS, L.: Stereospecific interaction between narcotic analgesics and synaptic plasma membrane fraction of rat cerebral cortex. Acta Pharmacol. Toxicol. **32:** 317-329, 1973.

THOMPSON, R. C., MANSOUR, A., AKIL, H., AND WATSON, S. J.: Cloning and pharmacological characterization of a rat μ opioid receptor. Neuron **11:** 903-913, 1993.

TIVE L. A., PICK, C. G., PAUL, D., ROQUES, B. P., GACEL, G. A., AND PASTERNAK, G. W.: Analgesic potency of TRIMU-5: a mixed μ2 opioid receptor agonist/μ1

opioid receptor antagonist. Eur. J. Pharmacol. 216: 249-255, 1992.
TOTH, G., KRAMER, T. H., KNAPP, R., LUI, G., DAVIS, P., BURKS, T. F., YAMAMURA, H. I., AND HRUBY, V. J.: [D-Pen^2, D-Pen^5]enkephalin analogues with increased affinity and selectivity for δ opioid receptors. J. Med. Chem. 33: 249-253, 1990.
TSENG, L. F., TSAI, J. H. H., COLLINS, K. A., AND PORTOGHESE, P. S.: Spinal delta(2)-, but not delta(1)-, mu, or kappa-opioid receptors are involved in the tail-flick inhibition induced by beta-endorphin from nucleus raphe obscurus in the pentobarbital-anesthetized rat. Eur. J. Pharmacol. 277: 251-256, 1995.
TSU, R. C., CHAN, J. S. C., AND WONG, Y. H.: Regulation of multiple effectors by the cloned δ-opioid receptor: stimulation of phospholipase C and type II adenylyl cyclase. J. Neurochem. 64: 2700-2707, 1995.
TWITCHELL, W. A., AND RANE, S. G.: Nucleotide-independent modulation of a Ca^{2+}-dependent K^+ channel current by a μ-type opioid receptor. Mol. Pharmacol. 49: 793-798, 1994.
UHL, G. R., CHILDERS, S., AND PASTERNAK, G.: An opiate-receptor gene family reunion. Trends Neurosci. 17: 89-93, 1994.
VANDERAH, T., TAKEMORI, A. E., SULTANA, M., PORTOGHESE, P. S., MOSBERG, H. I., HRUBY, V. J., HAASETH, R. C., MATSUNAGA, T. O., AND PORRECA, F.: Interaction of [D-Pen^2, D-Pen^5]enkephalin and [D-Ala^2, Glu^4]deltorphin with δ-opioid receptor subtypes in vivo. Eur. J. Pharmacol. 252: 133-137, 1994.
VANHOUTTE, P. M., HUMPHREY, P. P. A., AND SPEDDING, M.: X. International Union of Pharmacology recommendations for nomenclature of new receptor subtypes. Pharmacol. Rev. 48: 1-2, 1996.
VAUGHN, L. K., KNAPP, R. J., TOTH, G., WAN, Y. P., HRUBY, V. J., AND YAMAMURA, H. I.: A high affinity highly selective ligand for delta opioid receptor: [^{3}H] [D-Pen^2-pcl Phe^4-D-Pen^5] enkephalin. Life Sci. 45: 1001-1008, 1989.
VAUGHT, J. L., MATHIASEN, J. R., AND RAFFA, R. B.: Examination of the involvement of supraspinal and spinal mu and delta opioid receptors in analgesia using the mu receptor deficient CXBK mouse. J. Pharmacol. Exp. Ther. 245: 13-16, 1988.
VON VOIGTLANDER, P. F., LAHTI, R. A., AND LUDENS, J. H.: U-50,488H, a selective and structurally novel non-mu (kappa) opioid agonist. J. Pharmacol. Exp. Ther. 224: 7-12, 1983.
VON VOIGTLANDER, P. F., AND LEWIS, R. A.: Analgesic and mechanistic evaluation of spiradoline, a potent kappa opioid. J. Pharmacol. Exp. Ther. 246: 259-262, 1988.
WAKSMAN, G., HAMEL, E., FOURNIÉ-ZALUSKI, M. C., AND ROQUES, B. P.: Autoradiographic comparison of the distribution of the neutral endopeptidase "enkephalinase" and of mu and delta opioid receptors in rat brain. Proc. Natl. Acad. Sci. USA 83: 1523-1527, 1986.
WANG, H., PELAPRAT, D., ROQUES, B. P., VANHOVE, A., CHI, Z. Q., AND ROSTÈNE, W.: [^{3}H]Ohmefentanyl preferentially binds to μ-opioid receptors but also labels σ-sites in rat brain sections. Eur. J. Pharmacol. 193: 341-350, 1991.
WANG, J. B., IMIA, Y., EPPLER, C. M., GREGOR, P., SPIVAK, C. E., AND UHL, G. R.: μ Opiate receptor: cDNA cloning and expression. Proc. Natl. Acad. Sci. USA 90: 10230-10234, 1993.
WANG, J. B., JOHNSON, P. S., IMAI, Y., PERSICO, A. M., OZENBERGER, B. A., EPPLER, C. M., AND UHL, G. R.: cDNA cloning of an orphan opiate receptor gene family member and its splice variant. FEBS Lett. 348: 75-79, 1994a.
WANG, J. B., JOHNSON, P. S., PERSICO, A. M., HAWKINS, A. L., GRIFFIN, C. A., AND UHL. G. R.: Human μ opiate receptor: cDNA and genomic clones, pharmacologic characterization and chromosomal assignment. FEBS Lett. 338: 217-222, 1994b.
WANG, J. B. JOHNSON, P. S., WU, J. M., WANG, W. F., AND UHL, G. R.: Human kappa opiate receptor second extracellular loop elevates dynorphin's affinity for human mu/kappa chimeras. J. Biol. Chem. 269: 42: 25966-25969, 1994c.
WANG, L., AND GINTZLER, A. R.: Bimodal opioid regulation of cyclic AMP formation: implications for positive and negative coupling of opiate receptors to adenylyl cyclase. J. Neurochem. 63: 1726-1730, 1994.
WARD, S. J., FRIES, D. S., LARSON, D. L., PORTOGHESE, P. S., AND TAKEMORI, A. E.: Opioid receptor binding characteristics of the non-equilibrium mu antagonist, beta-funaltrexamine (beta-FNA). Eur. J. Pharmacol. 107: 323-330, 1985.
WICK, M. J., MINNERATH, S. R., LIN, X. Q., ELDE, R., LAW, P. Y., AND LOH, H. H.: Isolation of a novel cDNA encoding a putative membrane receptor with high homology to the cloned mu, delta, and kappa opioid receptors. Mol. Brain Res. 27: 37-44, 1994.
WILD, K. D., CARLISI, V. J., MOSBERG, H. I., BOWEN, W. D., PORTOGHESE, P. S., SULTANA, M., TAKEMORI, A. E., HRUBY, V. J., AND PORRECA, F.: Evidence for a single functional opioid delta receptor subtype in the mouse isolated vas deferens. J. Pharmacol. Exp. Ther. 264: 831-838, 1993a.
WILD, K. D., FANG, L., MCNUTT R. W., CHANG, K. J., TOTH, G., BORSODI, A., YAMAMURA, H. I., AND PORRECA, F.: Binding of BW 373U86, a non-peptidic δ opioid receptor agonist, is not regulated by guanine nucleotides and sodium. Eur. J. Pharmacol. Mol. Pharmacol. 246: 289-302, 1993b.
WILLIAMS, J. T., AND NORTH, R. A.: Opiate-receptor interactions on single locus coeruleus neurones. Mol. Pharmacol. 26: 489-497, 1984.
WIMPEY, T. L., AND CHAVKIN, C.: Opioids activate both an inward rectifier and a novel voltage-gated potassium conductance in the hippocampal formation. Neuron 6: 281-289, 1991.
WUARIN, J. P., AND DUDEK, F. E.: Direct effects of an opioid peptide selective for μ-receptors: intracellular recordings in the paraventricular and supraoptic nuclei of the guinea-pig. Neuroscience 36: 291-298, 1990.
WUSTER, M., SCHULZ, R., AND HERZ, A.: Specificity of opioids towards the μ-, δ- and ε- opiate receptors. Neurosci. Lett. 15: 193-198, 1979.
XIE, G., MIYAJIMA, A., AND GOLDSTEIN, A: Expression cloning of cDNA encoding a seven helix receptor from human placenta with affinity for κ opioid ligands. Proc. Natl. Acad. Sci. USA 89: 4124-4128, 1992.
XIE, G. X., MENG, F., MANSOUR, A., THOMPSON, R. C., HOVERSTEN, M. T., GOLDSTEIN, A., WATSON, S. J., AND AKIL, H.: Primary structure and functional expression of a guinea pig κ opioid (dynorphin) receptor. Proc. Natl. Acad. Sci. USA 91: 3779-3783, 1994.
XU, H., CHEN, J., AND CHI, Z. Q.: Ohmefentanyl—a new agonist for μ-opiate receptor. Sci. Sin. B 28: 504-511, 1985.
XU, H., GOODMAN, C. B., PARTILLA, J. S., NI, Q., KAYAKIRI, H., RICE, K. C., AND ROTHMAN, R. B.: 6β-[^{125}Iodo]-3,14-dihydroxy-17-methyl-4,5α-epoxymorphinan ([^{125}I]IOXY-AGO): a potent and selective radioligand for opioid μ receptors. Synapse 19: 105-111, 1995.
XUE, J. C., CHEN, C., ZHU, J., KUNAPULI, S., DERIEL, J. K., YU, L., AND LIU-CHEN, L. Y.: Differential binding domains of peptide and non-peptide ligands in the cloned rat κ opioid receptor. J. Biol. Chem. 269: 30195-30199, 1994.
YAMAMURA, M. S., HORVATH, R., TOTH, G., OTVOS, F., MALATYNSKA, E., KNAPP, R. J., PORRECA, F., HRUBY, V. J., AND YAMAMURA, H. I.: Characterization of [^{3}H]naltrindole binding to δ-opioid receptors in rat brain. Life Sci. 50: PL119–PL124, 1992.
YASUDA, K., ESPINOSA, R., TAKEDA, J., LE BEAU, M., AND BELL, G. I.: Localization of the kappa opioid receptor gene to human chromosome band 8q11.2. Genomics 19: 596-597, 1994.
YASUDA, K., RAYNOR, K., KONG, H., BREDER, C. D., TAKEDA, J., REISINE, T., AND BELL, G. I.: Cloning and functional comparison of kappa and delta opioid receptors from mouse brain. Proc. Natl. Acad. Sci. USA 90: 6736-6740, 1993.
YEADON, M., AND KITCHEN, I.: Multiple opioid receptors mediate the respiratory depressant effects of fentanyl-like drugs in the rat. Gen. Pharmacol. 21: 655-664, 1990.
YOSHINO, H., NAKAZAWA, T., ARAKAWA, Y., KANEKO, T., TSUCHIYA, Y., MATSUNAGA, M., ARAKI, S., IKEDA, M., YAMATSU, K., AND TACHIBANA, S.: Synthesis and structure-activity relationships of dynorphin A(1-8) amide analogues. J. Med. Chem. 33: 206-212, 1990.
YU, V., RICHARDS, M. L., AND SADEE, W.: A human neuroblastoma cell line expresses mu and delta opioid receptor sites. J. Biol. Chem. 261: 1065-1070, 1986.
ZAGON, I. S., GIBO, D. M., AND MCLAUGHLIN, P. J.: Zeta, a growth related opioid receptor in developing rat cerebellum: identification and characterization. Brain Res. 551: 28-35, 1991.
ZAJAC, J. M., GACEL, G., PETIT, F., DODEY, P., ROSSIGNOL, P., AND ROQUES, B. P.: Enkephalin Tyr-D-Thr-Gly-Phe-Leu-Thr: a new highly potent and fully specific agonist for opiate delta receptors. Biochem. Biophys. Res. Commun. 111: 390-397, 1983.
ZASTAWNY, R. L., GEORGE, S. R., NGUYEN, T., CHENG, R., TSATSOS, J., BRIONES-URBINA, R., AND O'DOWD, B. F.: Cloning, characterization, and distribution of a μ-opioid receptor in rat brain. J. Neurochem. 63: 2099-2105, 1994.
ZHANG, S. W., AND YU, L. C.: Identification of dynorphins as endogenous ligands for an opioid receptor-like orphan receptor. J. Biol. Chem. 270: 22772-22776, 1995.
ZUKIN, R. S., EGHBALI, M., OLIVE, D., UNTERWALD, E. M., AND TEMPEL, A.: Characterization and visualization of rat and guinea pig brain kappa opioid receptors: evidence for κ1 and κ2 opioid receptors. Proc. Natl. Acad. Sci. USA 85: 4061-4065, 1988.