NIDA Res Monogr. 1998 Mar;178:440-66.

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INTRODUCTION

One of the key missions of the National Institute on Drug Abuse is the development of drugs for the treatment of drug abuse. Although there are presently three drugs approved for treatment of opiate narcotic addiction (methadone, naltrexone, and LAAM), second and third generation compounds are desired. Because of the absence of any clinically used medications, of particular importance is the identification and development of treatment compounds for the psychostimulant cocaine.

The Medications Development Division at NIDA has established an Opiate Treatment Discovery Program (OTDP), and a Cocaine Treatment Discovery Program (CTDP) with the purpose of using simple preclinical tests for the identification and evaluation of compounds that may be of use in the treatment of opiate narcotic and cocaine abuse. Tests in these programs include *in vitro* binding and biochemical assays, and *in vivo* pharmacological studies, designed to inexpensively select the most promising compounds for further evaluation and possible development as treatment medications. The strategy chosen was to test a large number of unknown compounds at molecular targets (receptors or transporters) that may be involved in the addiction process for opiates and cocaine.

The potential sites for treatment of opiate addiction have been reasonably well defined over the past several years. The approved medications act as either very long-lasting μ opiate

agonists (methadone and LAAM), or a μ opiate antagonist (naltrexone). In addition, μ partial agonists, such as buprenorphine, have had indications of some usefulness in preclinical studies, and clinical trials (Kosten et al., 1992). Accordingly, for the OTDP, the binding affinity and activity of a large number of opiate compounds have been tested at μ-, δ-, and κ-opiate receptors. Binding studies were originally conducted in guinea pig brain membranes, and subsequent studies have been carried out in CHO cells transfected with human receptors. Activity has been determined using the *in vitro* bioassays guinea pig ileum (GPI) and mouse vas deferens (MVD). Additional studies have been carried out by measuring stimulation of [^{35}S]GTPγS binding in transfected cells.

The sites of action for potential cocaine treatment medications are significantly less well defined. Cocaine is a local anesthetic that has pharmacological actions throughout the body. However, the CNS stimulant actions of cocaine are known to be mediated by its ability to block the reuptake of the biogenic amines dopamine, norepinephrine, and serotonin (5-hydroxytryptamine, 5-HT) (Ritz et al, 1990, Reith, 1988). The addictive property of cocaine appears to be related to its ability to block dopamine reuptake (Kuhar et al., 1988). Accordingly, cocaine mediates its effects by increasing synaptic levels of dopamine and the other biogenic amines. Based upon the success of opiate addiction medications, potential cocaine treatment medications could include long-lasting cocaine analogs, long-lasting dopamine receptor agonists, dopamine receptor antagonists, dopamine receptor partial agonists. Unfortunately, unlike opiates, for which we know the μ receptor mediates the rewarding actions, we don't know which of the five dopamine receptors mediate the rewarding aspects of cocaine administration.

The objective of one CTDP contract was to evaluate a large number of compounds that interact with biogenic amine receptors for potential cocaine treatment medications. We characterized compounds at dopamine (D_1, D_2, and D_3), 5-HT (5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_3), phencyclidine (PCP), and sigma (σ) binding sites. The characterization included both determination of binding affinities and, where possible, an evaluation of the agonist or antagonist potencies of compounds at the dopamine and 5-HT receptors. Activity at D_1 receptors has been determined by measuring stimulation of cAMP accumulation. Activity at D_2 and D_3 was determined by measuring stimulation of mitogenic activity. Activity at 5-HT_{2A} receptors was measured in the rat aorta spiral, *in vitro*, and activity at the 5-HT_3 receptor was measured in the GPI.

Prior to the study of unknowns at each site discussed above, our binding and functional assays were validated by the characterization of a large number of known standard

compounds. In this manuscript, we will list binding affinities and activities of standard compounds derived for both OTDP and CTDP programs.

METHODS

Receptor Binding

Serotonin Receptors

Human 5-HT_{1A} receptors on HA7 cells were donated by Dr. Marc Caron. NIH-3T3-GF6 cells containing rat 5-HT_{2A} receptors and NIH-3T3-Pø cells containing rat 5-HT_{2C} receptors were obtained from Dr. David Julius. Each cell line is grown in Dulbecco's minimum essential medium (DMEM) containing 10% fetal calf serum, 0.05% pen-streptomycin, and 400 μg/ml G418. The cells are scraped from the 100 × 20 mm plates and centrifuged at 500 × *g* for 5 min. The pellet is homogenized in 50 mM Tris-HCl, pH 7.7, with a polytron, centrifuged at 27,000 × *g*, and resuspended in the same buffer, washed once and resuspended in 25 mM Tris-HCl containing 100 μM ascorbic acid and 10 μM nialamide at pH 7.4 for 5-HT_{1A} receptors, 25 mM Tris-HCl, pH 7.7 for 5-HT_{2A} receptors, and 50 mM Tris-HCl, pH 7.7, 4 mM $CaCl_2$, 10 μM pargyline, and 0.1% ascorbic acid for 5-HT_{2C} receptors. The binding assays contain [^{3}H]8-OH-DPAT (0.5 nM final concentration), and 30 µg protein/tube, [^{3}H]ketanserin (0.40 nM final concentration) with 30 µg protein/tube, or [^{3}H]mesulergine (0.4 nM final concentration), and 8 µg protein/tube, in a volume of 1.0 ml for 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors respectively. The tubes are incubated at 25˚C for 60 min before filtration.

5-HT_3 receptors are found in the neuroblastoma x glioma hybrid cells NG108-15, which are grown in DMEM with HAT supplement and 10% fetal calf serum. The cell membranes are prepared as described above and resuspended in 25 mM Tris-HCl, pH 7.7 with 1 mM EDTA. The assay is performed in 0.5 ml with [^{3}H]GR65630 (1.6 nM final concentration), and 0.13 mg protein to each tube. The tubes are then incubated at 25˚C for 45 min. Nonspecific binding is defined by 1 μM of zacopride. Filters are soaked in 0.1% polyethyleneimine (PEI) before filtering.

Dopamine Receptors

Human D_1 receptors in L cells, obtained from, Dr. David Grandy, are grown and prepared as described for the HA7 cells. The final pellet is resuspended in 50 mM Tris-HCl containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$, pH 7.7. Binding is

conducted as described above using [^{3}H]SCH 23,390 (0.18 nM final concentration) and 70 μg protein/ tube. Human D_2- and D_3-receptor-containing CHOp- cells were obtained from Dr. Robert MacKenzie, and are grown in α minimum essential medium (α MEM) containing 10% fetal calf serum, 0.05% penicillin-streptomycin, and 600 μg/ml G418. Membranes are prepared as described above. The final pellet is resuspended in 50 mM Tris containing 120 mM NaCl, 5 mM of KCl, 1.5 mM $CaCl_2$, 4 mM of $MgCl_2$, and 1 mM EDTA, pH 7.4. The binding incubation is in 2.0 ml and contains 30 μg protein/tube, and [^{3}H]YM-09151-2 (0.2 nM final concentration). Filters are soaked in 0.1% PEI before filtering.

μ, δ, and κ Opioid Receptors

Receptor binding studies were initially conducted on Hartley guinea pig brain membranes. Guinea pigs were decapitated and the brains quickly removed and weighed, then homogenized in 50 mM Tris HCl, pH 7.5, using a Polytron homogenizer. The homogenate was centrifuged at 40,000 × *g* for 15 min, rehomogenized, and centrifuged once more. The final pellet was resuspended in Tris HCl, pH 7.5, at a final concentration of 6.67 mg original wet weight of tissue per milliliter.

Routine binding assays are conducted using [^{3}H]DAMGO, [^{3}H]Cl-DPDPE, [^{3}H]U69,593 for binding to μ, δ, κ receptors, respectively. Binding is conducted in a total volume of 2.0 ml, usually for 1 h at 25°C. Samples are filtered and counted as described above.

Binding was also conducted on cloned receptors. Human κ-opioid receptor-containing CHO cells were obtained from Dr. Lee-Yuan Liu-Chen, and are grown in DMEM with 10% fetal calf serum, 0.4 mg/ml G418, and 0.1% penicillin/streptomycin. Human μ-opioid receptor-containing CHO cells were obtained from Dr. George Uhl, and are grown in F12 medium containing 10% fetal calf serum and 0.4 mg/ml G418. Human δ-opioid receptor-containing CHO cells were obtained from Dr. Hank Yamamura, and are grown in F12 medium containing 10% fetal calf serum and 0.5 mg/ml hygromycin B. Cell membranes are prepared as described above. For binding, 30 μg protein is incubated in 50 mM Tris buffer pH 7.7, with approximately 0.5 nM of the radioligand. Incubation volume is 1.0 ml. Samples were filtered and counted as described above.

Functional Biochemical Assays

cAMP Production

C6 cells containing monkey D_1 receptor were obtained from Dr. Kim Neve, and were grown on 96-well plates in DMEM containing 10% FBS and 2 μg/ml of puromycin. D_1 receptors stimulate adenylyl cyclase, so for these receptors an increase in cAMP accumulation in intact cells is measured. When the cells in each well have reached confluence, the medium is removed and each well is rinsed with 0.1 ml of Krebs-HEPES buffer (130 mM of NaCl, 4.8 mM of KCl, 1.2 mM of KH_2PO_4, 1.3 mM of $CaCl_2$, 1.2 mM of $MgSO_4$, 25 mM of HEPES, and 10 mM of glucose, pH 7.3). The test drug is diluted in Krebs-HEPES buffer containing 0.1% ascorbic acid, 10 μM of pargyline, and 50 μM of 3-isobutyl-1-methylxantine (IBMX) and 0.1 ml is added to each well. The plates are preincubated for 20 min at 37°C with or without antagonist, then incubated for an additional 10 min with the test compound. After incubation, the medium is removed and 0.1 ml of 0.5 M formic acid is added, then the supernatant is removed and lyophilized. cAMP is quantitated using the protein kinase binding assay of Gilman (1970).

Stimulation of Mitogenesis

To measure D_2 and D_3 stimulation of mitogenesis (Chio et al., 1994), CHOp- cells are used in a 96-well plate containing approximately 5,000 cells/well. The cells are incubated at 37°C in α MEM with 10% FBS, 0.05% penicillin-streptomycin, and 200 μg/ml Geneticin (G418 sulfate). After 48 h, the wells are rinsed twice with 100-μl aliquots of serum-free α MEM and incubated for 24 h at 37°C in serum-free α MEM. The medium is then removed and replaced with 90 μl of serum-free α MEM and 10 μl of drug in sterile water. After another 24 h of incubation at 37°C, 0.25 μCi of [^{3}H]thymidine is added to each well. The cells are incubated for 2 h at 37°C. Then, 10 μl/well of 10× trypsin is added to remove the cells, and the plate is filtered using a Tomtec cell harvester, and counted in a Betaplate Reader (Wallac).

[^{35}S]GTPγS Binding

[^{35}S]GTPγS binding is used to measure activity of μ, δ, and κ opioid receptors. Binding is conducted basically as described by Traynor and Nahorski (1995). Cells are prepared as described above, and suspended in Buffer A, containing 20 mM HEPES, 10 mM $MgCl_2$, 100 mM NaCl, pH 7.4. For the binding assay, membranes (15 μg protein) are incubated with [^{35}S]GTPγS (50 pM), GDP (usually 10 μM), and the desired compound, in a total volume of 1 ml, for 60 min at 25°C. Samples are filtered over glass fiber filters and counted as described for the binding assays above. Dose-response curves with the full agonists DAMGO, DPDPE, and U69,593 are determined in each experiment to identify full and partial agonist compounds at μ-, δ-, and κ-opioid receptors, respectively.

In Vitro Functional Smooth muscle bioassays

5-HT_{2A} Receptor

Rat Aorta Spiral (RAS) Tissue Preparation

Male albino Wistar rats (200-300 g body weight) are sacrificed and their aortas quickly removed, cleaned, and cut into a spiral. The spiral is mounted in an 8-ml water-jacketed tissue bath containing Krebs-bicarbonate solution (118 mM NaCl, 2.5 mM $CaCl_2$, 4.7 mM KCl, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, and 11.5 mM glucose). The spiral is first incubated with oxygenated (5% CO_2 in oxygen) Krebs solution at 37°C under 2.0 g tension for 30 min, then an additional 30 min in the presence of 500 μM of pargyline, a monoamine oxidase inhibitor, and 10 μM of benextramine tetrahydrochloride, an α_1-adrenoceptor inhibitor (Marin et al., 1981). Excess unreacted pargyline and benextramine are then removed from the bath by flushing the system several times with the Krebs solution.

Serotonin (5-HT) Standard Curve

For standardization purposes, the spiral is cumulatively contracted with increasing concentrations of 5-HT in the presence of 10^{-4} M ascorbic acid. The 5-HT-induced contractions are recorded using an isometric transducer (Metrigram) coupled to a Gould multichannel polygraph.

Agonist Determinations

A concentration-response curve is generated for the test compound, and an ED_{50} (agonist concentration that produces half of the maximum contraction attainable by the agonist) value determined. To verify the test compound's agonist activity at 5-HT_{2A} receptors, assays are conducted in the presence of 100 nM ketanserin.

Antagonist Determinations

Test compounds that do not produce a contraction of the spiral on their own are tested for 5-HT_{2A} antagonist activity. The test compound is incubated with the spiral for 30 min, and then the 5-HT standard curve is repeated in the presence of the drug. Antagonist activities are calculated for each single tissue from full concentration-response curves before and after addition of a single antagonist concentration. At least three different concentrations are used, and only one antagonist concentration is tested on each tissue. pA_2 values are determined from Schild plots (Schild, 1949) using a statistical analysis program developed by B. Eynon (SRI International).

5-HT$_3$ Receptor

Longitudinal Muscle Strip of Guinea Pig Ileum (GPI) Preparation

Male Hartley guinea pigs weighing 350-400 g are decapitated and the small intestine removed. The longitudinal muscle, with the myenteric plexus attached, is gently separated from the underlying circular muscle by the method of Paton and Vizi (1969). The muscle strips are mounted in an 8-ml, water-jacketed organ bath containing Krebs-bicarbonate solution. The tissues are kept at 37˚C and bubbled with 5% CO_2 in oxygen. An initial tension of 1.0 g is applied to the strips. The tissues are equilibrated for 60 min before the start of the experiments. The contractions are recorded as described above.

Agonist and Antagonist Determinations

Concentration-response curves are constructed with the selective 5-HT$_3$ agonist 2-methyl-5-HT at concentrations from 1 to 100 μM. Individual doses are given 20 min apart. Then the test compound is injected into the tissue bath. If the compound contracts the GPI preparation, a concentration-response curve is constructed and the ED_{50} value is determined both in the absence and presence of 100 nM ICS 205-930, a selective 5-HT$_3$ antagonist. Antagonist determinations using the Schild method are conducted as described above for the 5-HT$_{2A}$ receptor, using the selective 5-HT$_3$ agonist 2-methyl-5-HT.

Opioid Receptors

Longitudinal Muscle Strip of Guinea Pig Ileum (GPI)

The tissue is prepared as described above for the 5-HT$_3$ receptor except that the muscle strip is stimulated for 60 min before the start of each experiment. Field electrical stimulation is delivered through platinum wire electrodes positioned at the top and bottom of the organ bath and kept a fixed distance apart (3.5 cm). The upper electrode is a ring 4 mm in diameter. The parameters of rectangular stimulation are supramaximal voltage, 1-ms impulse duration at 0.1 Hz. A Grass S-88 electrostimulator is used for stimulation.

Electrically Stimulated Mouse Vas Deferens (MVD)

Swiss-Webster mice weighing 30-35 g are used. The vasa deferentia are prepared according to the method of Hughes et al. (1975), bathed at 31˚C in Mg^{2+} -free Krebs solution, and bubbled with a mixture of oxygen and CO_2 (95:5). An initial tension of 200-350 mg is used. The parameters of field stimulation have been modified slightly from the original description (Ronai et al., 1977); paired shocks of 100-ms delay between supramaximal rectangular pulses of 1-ms duration are delivered at a rate of 0.1 Hz.

Kinetics

The agonist potencies of test compounds are determined from concentration-response curves and characterized by their IC_{50} values. IC_{50} is defined as the concentration of the agonist that causes 50% inhibition of the electrically induced contractions.

Compounds with antagonist activity are characterized by the equilibrium dissociation constant (K_e) calculated from the following equation:

$$K_e = a/(DR - 1)$$

where "a" is the nanomolar concentration of antagonist and DR is the virtual shift of the agonist concentration-response curve to the right in the presence of a given concentration of antagonist. In the case of mixed agonist-antagonist compounds, the dose ratios are determined by the "single-dose method" introduced by Kosterlitz and Watt (1968).

A standard ratio with respect to a reference compound is also determined. The reference compound for μ- agonists is DAMGO, that for κ- agonists is U69,593 and that for δ-agonists is DPDPE. Their K_e values with the respective/selective antagonists are taken as 1.0.

RESULTS AND DISCUSSION

Cocaine Treatment Discovery Program

Binding affinities derived at serotonin and dopamine receptors are shown in Table 1. Affinities are given as $K_{0.5}$ values ± Standard Deviation of, in general, two experiments. Also shown are Hill coefficients derived in those two experiments. $K_{0.5}$ values are derived from the Cheng-Prusoff equation: $K_i = IC_{50}/(1 + [L]/K_d)$. This equation is only strictly valid for inhibition curves with Hill coefficients of 1.0. Because binding experiments have generally used [^{3}H]antagonists that bind to high and low affinity conformations, Hill coefficients of unlabeled agonists are often less than 1.0, necessitating the designation $K_{0.5}$.

Activity at D_1 receptors was determined by measuring agonist-stimulated increase in cAMP accumulation. One problem encountered with this assay was that compounds known to be partial agonists exhibited full agonist activity when measured in the D_1 receptor-containing cells first obtained. Subsequently, we obtained C6 cells containing either high or low copy number of the monkey D_1 receptor. As seen in Table 2, both cell lines could be

successfully used to measure an agonist-stimulated increase in cAMP accumulation. However, in the high expressing cells both full agonists dopamine and SKF-81297, and partial agonists SKF-38393 and SKF-77434 acted as full agonists. In the low expressing cells, both partial agonists clearly produced significantly less cAMP than the full agonists tested. In addition, as would be predicted for low expressing cells with no receptor reserve, each of the agonists was less potent, as demonstrated by a higher value for the EC_{50}. To identify partial agonist compounds, all further experiments were conducted on the low expressing cells.

Activity at D_2 and D_3 receptors was determined by measuring stimulation of mitogenic activity (Table 3). All experiments were done in comparison to stimulation of mitogenesis by the D_2/D_3 full agonist quinpirole, conducted on the same day. In this way percent maximal stimulation could be accurately determined. This method has been useful for the identification of full and partial agonists at D_2 and D_3 receptors.

Functional activity at 5-HT_{2A} and 5-HT_3 receptors have been determined using smooth muscle bioassays (Table 4), as described in Methods. Most of the standards tested have been antagonists at one or both of the 5-HT receptors. For each antagonist, K_e values were determined by full Schild analysis.

Opiate Treatment Discovery Program

Table 5 shows values derived from binding to the μ-, δ-, and κ-opioid receptors. At the beginning of the contract, all opioid binding studies were conducted on guinea pig brain membranes. With the cloning of the opioid receptors, and the subsequent availability of human receptors, recent experiments were conducted on human opioid receptor-containing CHO cells. A comparison of guinea pig brain and human receptor binding indicates a very close correlation between the two species. These affinities are listed as K_i values and were also derived from the Cheng-Prusoff equation. Because [^{3}H]agonists were used in all of these binding experiments, Hill coefficients were uniformly close to 1.0, and the designation K_i is appropriate.

Historically, smooth muscle bioassays have been used extensively for the characterization of opioid compounds. The guinea pig ileum can be used for characterization of compounds at μ and κ receptors. The mouse vas deferens has μ, δ, and κ receptors. However, it is apparently highest in δ receptors, and has often been used to characterize compounds at the δ opioid receptor. The activities of standard compounds at μ and κ receptors in the GPI,

and at δ receptors in the MVD are shown in Table 6. The values shown are: IC_{50}, concentration at which the compounds inhibits 50% of the magnitude of the electrically-induced contractions; the dose ratio (DR), the shift in IC_{50} in the presence of 200 nM CTAP, 1 nM Nor-BNI, or 1 nM naltrindole; the K_e of the antagonist, and the ratio with respect to the prototypical agonist. Together, these values show the activity of each compound, as well as the selectivity of each compound.

Table 7 shows a biochemical method for determining activity and potency of opioid compounds, stimulation of [^{35}S]GTPγS binding in membranes from cells transfected with human μ, δ, or κ receptors. As with the mitogenesis assay, a standard prototypical agonist (DAMGO, DPDPE, and U69,693 for μ, δ, and κ receptors) is tested in every experiment so that percent maximal stimulation, with respect to the standard agonist, can be determined for each compound. In general, values determined in the GTPγS assays correspond quite well with the data derived from the smooth muscle bioassays. Compounds that are listed as flat at any particular site either are antagonists, or have low affinity for that particular site. K_e values of antagonists have not yet been determined in this assay. The advantages of this assay are that one measures activity in tissue containing a single receptor type, so that the use of selective antagonists is not necessary to clearly identify the site of action. In addition, GTPγS binding seems to clearly identify the efficacy of compounds. For instance, compounds such as buprenorphine and pentazocine can easily be identified as partial agonists.

The values listed in each of these tables have also been incorporated into NIDA's Medications Development Database. NIDA's OTDP and CTDP programs are still active, and we encourage the delivery of compounds into these programs for the continued identification of potential addiction medications.

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ACKNOWLEDGMENTS

This work was supported by NIDA contracts N01DA-3-8302 and N01DA-4-8307

Table 1

AFFINITIES OF STANDARD COMPOUNDS AT DOPAMINE AND SEROTONIN RECEPTORS

	$K_{0.5}$ ± S.D. (nM) and Hill Coefficient						
Cold Ligand	D_1 [^{3}H]SCH 23390	D_2 [^{3}H]YM-09151-2	D_3 [^{3}H]YM-09151-2	5-HT_{1A} [^{3}H]8-OH-DPAT	5-HT_{2A} [^{3}H]Ketanserin	5-HT_{2C} [^{3}H]Mesulergine	5-HT_3 [^{3}H]GR65630
d-Amphetamine	>10,000	>10,000	>10,000	6606 ± 1327 0.9	>10,000	>10,000	>10,000
SKF-82958	22 ± 3.0 0.7	136 ± 64.3 0.6	68 ± 5.9 1	1781 ± 383 1	1295 ± 19.1 0.9	4626 ± 764 1	>10,000
Apomorphine	400 ± 55.3 0.8	42 ± 8.2 1.6	27 ± 6.3 1	4929 ± 3048	4377 ± 1217 1	1103 ± 345 1	>10,000
(+)Bromocriptine	2070 ± 392 1.3	8.2 ± 1.1 1.2	7.1 ± 1.5 1.5	25 ± 14.7 2.1	117 ± 8.4 1.4	4674 ± 256	>10,000
(+)Butaclamol	4.2 ± 1.55 1.1	1 ± 0 1.3	1.4 ± 0 0.9	528 ± 162 0.7	5.2 ± 1.2 1.1	1064 ± 338 1	>10,000
Carbamazepine	>10,000	>10,000		>10,000	>10,000	>10,000	>10,000
CGS-12066B	1529 ± 700 1.4	1739 ± 343 1.5	580 ± 281 1.4	44 ± 0.45 1.1	3134 ± 1347 1.5	6601 ± 574 1.1	>10,000
Chlorpromazine	44 ± 1.4 0.8	3 ± 0.65 0.8	1.8 ± 0.25 1.1	3115 ± 260 0.8	3.6 ± 1.7 0.8	39 ± 2.4 1.1	820 ± 13.9 3.3
Clomipramine	219 ± 5.1 1	162 ± 2 0.8	30 ± 5.6 1	>10,000	11 ± 2.6 0.8	52 ± 13.4 1.1	985 ± 302 1
Cocaine	>10,000*	>10,000		>10,000	>10,000	>10,000	>10,000
Cyproheptadine	117 ± 14.6 0.9	112 ± 53 1	8 ± 2.8 0.5	59 ± 23.6 0.9	0.7 ± 0.1 1.2	15 ± 3.7 1	228 ± 23.3 1.1
Deprenyl	>10,000	>10,000		>10,000	5638 ± 1350 1.1	>10,000	>10,000
Desipramine	5465 ± 461 0.6	1561 ± 237 1.6		>10,000	106 ± 8.3 0.9	748 ± 57.4 1.1	4402 ± 385 1.6
Dihydroergotamine	2779 ± 186 1	5 ± 0.8 1.1	16 ± 3.4 1	1.5 ± 0.55 1.6	38 ± 14.1 1.7	298 ± 115 1	>10,000

Table 1 (continued)

AFFINITIES OF STANDARD COMPOUNDS AT DOPAMINE AND SEROTONIN RECEPTORS

	$K_{0.5}$ ± S.D. (nM) and Hill Coefficient						
Cold Ligand	D_1 [^{3}H]SCH 23390	D_2 [^{3}H]YM-09151-2	D_3 [^{3}H]YM-09151-2	5-HT_{1A} [^{3}H]8-OH-DPAT	5-HT_{2A} [^{3}H]Ketanserin	5-HT_{2C} [^{3}H]Mesulergine	5-HT_3 [^{3}H]GR65630
L-DOPA	>10,000	>10,000		>10,000	>10,000	>10,000	>10,000
Dopamine	4470 ± 1598 0.8	422 ± 9.2 0.7	20 ± 1.2 0.6	8248 ± 1454 1	>10,000	>10,000	>10,000
8-OH-DPAT	>10,000	1788 ± 265		6.9 ± 2.6 0.6	>10,000	>10,000	>10,000
S(-)-Eticlopride	>10,000	0.1 ± 0 1.3	0.1 ± 0.05 0.8	1790 ± 160 1.2	705 ± 76.8 0.8	>10,000	>10,000
cis(Z)Flupentixol	3 1.2	1.5 ± 0.2 0.9	1.7 ± 1.3 1.1	8028 ± 159 0.6	13 ± 0.35 1.2	295 ± 15.5 1.2	>10,000
Fluphenazine	7 ± 1.4 1.2	0.9 ± 0.15 1	0.9 ± 0.1 1	2829 ± 1135 0.8	17 ± 1.8 1.3	1011 ± 331 1.1	>10,000
GBR-12909	>10,000	737 ± 231 1.1	109 ± 44.9 0.7	4677 ± 598 0.9	161 ± 10.2 1.1	>10,000	>10,000
GR-38032F	>10,000	>10,000		>10,000	>10,000	>10,000	15 ± 1.9 0.4
Haloperidol	58 ± 5.3 1	0.5 ± 0 1	2.1 ± 0.85 0.5	5084 ± 956 0.7	34 ± 5.2 0.7	>10,000	>10,000
Ibogaine	>10,000	>10,000		>10,000	>10,000	>10,000	>10,000
ICS 205-930	>10,000	>10,000		>10,000	5607 ± 240 1	>10,000	0.5 ± 0.25 0.9
Idazoxan	>10,000	>10,000		662 ± 216 1.1	>10,000	>10,000	>10,000
Imipramine	>10,000	726 ± 134 1.1	387 ± 108 0.9	>10,000	102 ± 8.0 1.1	106 ± 11.7 1.3	3651 ±238 0.7
Ketanserin	464 ± 90.2 1.1	>10,000		>10,000	1.6 ± 0.05 1	69 ± 16.4 1.3	>10,000

Table 1 (continued)

AFFINITIES OF STANDARD COMPOUNDS AT DOPAMINE AND SEROTONIN RECEPTORS

	$K_{0.5}$ ± S.D. (nM) and Hill Coefficient						
Cold Ligand	D_1 [^{3}H]SCH 23390	D_2 [^{3}H]YM-09151-2	D_3 [^{3}H]YM-09151-2	5-HT_{1A} [^{3}H]8-OH-DPAT	5-HT_{2A} [^{3}H]Ketanserin	5-HT_{2C} [^{3}H]Mesulergine	5-HT_3 [^{3}H]GR65630
MDL-72222	>10,000	>10,000		>10,000	2852 ± 1318 0.9	>10,000	14 ± 1.5 0.9
(-)MDA	>10,000	>10,000		>10,000	3296 ± 2104 0.5	2598 ± 1476 0.9	>10,000
(-)MDMA	>10,000	>10,000		>10,000	3911 ± 81.9 1	>10,000	>10,000
(+)Methamphetamine	>10,000	>10,000		>10,000	>10,000	>10,000	>10,000
2-Methyl-5-HT	>10,000	>10,000		3074 ± 72.2 0.9	>10,000	1367 ± 184 1.4	995 ± 80.0 1.1
Methylphenidate	>10,000	>10,000		>10,000	>10,000	>10,000	>10,000
Metoclopromide	>10,000*	64 ± 38.4 1	16 ± 5.1 0.7	>10,000	2063 ± 117 0.9	3859 ± 62.6 1.2	353 ± 62.3 1.3
Mianserin	426 ± 29.2 0.8	1274 ± 380 1.5		2592 ± 185 0.8	2.3 ± 0.05 1.1	11 ± 0.05 1.2	300 ± 72.8 1.1
NAN-190	4510 ± 911 1	47 ± 12. 0.8	3.4 ± 1.4 0.4	3 ± 1.8 0.8	549 ± 79.6 1.3	4117 ± 843 1	>10,000
Nomifensine	>10,000	>10,000		1183 ± 261 1	874 ± 203 1	>10,000	>10,000
Norepinephrine	>10,000	>10,000		>10,000	>10,000	>10,000	
Phentolamine	>10,000	>10,000		2151 ± 183 0.7	222 ± 33.6 1	459 ± 82.7 1	4294 ± 407 1.1
Pimozide	>10,000*	2.2 ± 0.45 1	2.3 ± 1.0 1.8	650 ± 62.2 1.2	49 ± 0.8 1.2	5787 ± 1679 1.3	3292 ± 108 3.2
S(-)-3-PPP	>10,000*	192 ± 11.4 1	262 ± 25.6 1.1	4174 ± 1706 0.4	5988 ± 797 1	>10,000	>10,000
(+/-)Propranolol	>10,000*	>10,000		272 ± 96.5 1	1047 ± 321 0.6	2457 ± 137 1	>10,000

Table 1 (continued)

AFFINITIES OF STANDARD COMPOUNDS AT DOPAMINE AND SEROTONIN RECEPTORS

	$K_{0.5}$ ± S.D. (nM) and Hill Coefficient						
Cold Ligand	D_1 [^{3}H]SCH 23390	D_2 [^{3}H]YM-09151-2	D_3 [^{3}H]YM-09151-2	5-HT_{1A} [^{3}H]8-OH-DPAT	5-HT_{2A} [^{3}H]Ketanserin	5-HT_{2C} [^{3}H]Mesulergine	5-HT_3 [^{3}H]GR65630
Quinpirole	>10,000	1185 ± 265 0.5	43 ± 19.7 1	1713 ± 285 1.2	>10,000	>10,000	>10,000
Quipazine	>10,000	>10,000		>10,000	92 ± 19.2 0.4	653 ± 58.8 1.2	3.2 ± 0.9 1.1
Reserpine	3849 ± 1297	586 ± 339 1.7	598 ± 51.7 3.9	1832 ± 694 1.7	3057 ± 231	3098 ± 267 2.1	>10,000
Ritanserin	933 ± 371 0.9	84 ± 46.2 1.1	24 ± 3 1.8	2919 ± 45.9 0.9	4.7 ± 1.6 1.1	11 ± 7.3 1.9	>10,000
R(+)SCH-23390	0.8 ± 0.1 1.1	431 ± 9.2 1.1	2421 ± 417 1.5	661 ± 172 0.6	12.2 ± 0.85 1	51 ± 3.8 0.8	3912 ± 1904 1.3
Serotonin	>10,000	>10,000		1.3 ± 0.15 1.1	96 ± 10.0 0.6	44 ± 5.8 0.7	292 ± 74.8 1
(+/-)SKF-38393	987 ± 56.7 0.9	>10,000	>10,000	3096 ± 0.35 0.8	6160 ± 1176 1	>10,000	>10,000
SKF-77434	73 ± 2.0 0.9	580 ± 25 0.6	10 ± 0.35 0.7	2107 ± 1084 1.7	2700 ± 78.6 1.3	3037 ± 387 1.1	
SKF-81297	112 ± 13.9 1.2	>10,000	792 ± 153 1.1	5516 ± 1888 1.4	3083 ± 977 1.2	1510 ± 781 1.4	
Spiperone	577 ± 50.8 1	0.1 ± 0 1.2	0.2 ± 0.05 1.5	61 ± 36.6 0.7	0.6 ± 0.15 1.1	4094 ± 1778 1.2	>10,000
Sulpiride	>10,000*	4.2 ± 1.8 0.8	15 ± 2.4 1	>10,000	>10,000	>10,000	>10,000
Tranylcypromine	>10,000	>10,000		5963 ± 412 0.7	>10,000	>10,000	>10,000
YM-09151-2	2602 ± 1066 1.99	0.1 ± 0.05 1.1	0.2 ± 0.1 1.5	15 ± 5.4 1.2	58 ± 5.3 0.7	3118 ± 2070 1.2	526 ± 7 0.9
Yohimbine	>10,000*	280 ± 52.1 1	2489 ± 521 1.1	642 ± 309 1.1	2258 ± 98.1 0.9	>10,000	>10,000
Zimelidine	>10,000*	1874 ± 38.6 0.9		>10,000	875 ± 329 1	482 ± 9.3 1.1	>10,000

Table 2

AGONIST POTENCIES FOR STIMULATION OF cAMP ACCUMULATION IN C6D1L (Low Expressor) AND C6D1H (High Expressor) CELLS

Compound	C6D1L		C6D1H	
	EC_{50} (nM)	% Max Stim.	EC_{50} (nM)	% Max. Stim.
Apomorphine	180	92		
Dopamine	52	100	2.3	100
SKF-38393	44	34	6.4	95
SKF-77434	22	10	5.6	89
SKF-81297	3.4	113	0.14	94
SKF-82958	5.2	88		

Table 3

AGONIST POTENCIES FOR STIMULATION OF MITOGENESIS IN CHOp-D_2 and D_3 CELLS

Compound	D_2 EC_{50} (nM)	% Max Stim	D_3 EC_{50} (nM)	% Max Stim
Apomorphine	11.00 ± 3.75	66	8.3 ± 2	100
(+)Bromocriptine	0.34 ± 0.2	81	23.0 ± 12	119
Dihydroergocristine	0.82 ± 0.015	99	22.0 ± 2	98
Dihydroergotamine	1.20 ± 0.48	59	3.1 ± 0.7	100
Dopamine	65.00 ± 15	90	6.1 ± 0.4	100
N-0437	0.45 ± 0.28	76	1.4 ± 0.4	90
Quinpirole	19.00 ± 16	100	8.4 ± 5.74	100
S(-)-3-PPP	64.00 ± 7	90		
SKF-82958	158.00 ± 56.5	52		
7-OH-DPAT	2.80 ± 0.55	102	1.0 ± 0.24	86
Terguride	0.28 ± 0.13	96	0.72 ± 0.06	88

Table 4

BIOASSAY RESULTS AT 5-HT_{2A} AND 5-HT_3 RECEPTORS

	RAT AORTA SPIRAL 5-HT_{2A}			GUINEA PIG ILEUM 5-HT_3		
COMPOUND	ED_{50} [μM] Agonist	DR with Ketanserin	K_e [nM] Antagonist	ED_{50} [μM] Agonist	DR with ICS 205-930	K_e [nM] Antagonist
d-Amphetamine				a		No inhibition from 10^{-8} to 10^{-5} M
(+)Bromocriptine			28.62 ± 4.65 (4)			
(+)Butaclamol	a		0.27 ± 0.10 (4)			
Chlorpromazine			4.04 ± 1.45 (5)			
Clomipramine			10.31 ± 4.87 (4)			
Cocaine	a		No inhibition from 10^{-8} to 10^{-5} M	a		1,517 ± 585 (7)
Cyproheptadine			0.017 ± 0.004 (4)			9.46 ± 4.04[b] (4)
Desipramine			225.23 ± 72.80 (4)			
Dihydroergotamine			1.11 ± 0.54 (5)			
cis(Z)Flupentixol	a		2.82 ± 1.33 (6)			
Fluphenazine			5.64 ± 2.47 (4)			
GBR-12909	a		41.84 ± 27.26 (7)			
Granisetron						3.85 ± 1.42 (8)

[a]No agonist activity was found from 10^{-9} to 10^{-5}M.

[b]There was a maximum depression at each concentrations, studied.

Table 4 (continued)

BIOASSAY RESULTS AT 5-HT_{2A} AND 5-HT_3 RECEPTORS

	RAT AORTA SPIRAL 5-HT_{2A}			GUINEA PIG ILEUM 5-HT_3		
COMPOUND	ED_{50} [μM] Agonist	DR with Ketanserin	K_e [nM] Antagonist	ED_{50} [μM] Agonist	DR with ICS 205-930	K_e [nM] Antagonist
GR-38032F						62.52 ± 16.0 (14)
Haloperidol	[a]		18.57 ± 4.02 (3)			
Imipramine			172.85 ± 74 (4)			
ICS 205-930						9.51 ± 1.13 (11)
Ketanserin			0.68 ± 0.31 (4)			
Mianserin			0.38 ± 0.18 (7)			352.84 ± 130.95[c] (3)
MDL 72222						272 ± 89 (6)
Phentolamine			552.19 ± 178 (8)			
Pimozide			8.61 ± 2.63 (5)			
Quipazine	0.302 ± 0.018 (3)			0.31 ± 0.04 (3)		
Ritanserin	[a]		0.026 ± 0.01 (4)			
R(+)SCH-23390	[a]		7.26 ± 1.72 (6)			

[a]No agonist activity was found.

[c]There was a maximum depression at 2.5×10^{-6} M concentration, studied.

Table 4 (concluded)

BIOASSAY RESULTS AT 5-HT_{2A} AND 5-HT_3 RECEPTORS

	RAT AORTA SPIRAL 5-HT_{2A}			GUINEA PIG ILEUM 5-HT_3		
COMPOUND	ED_{50} [μM] Agonist	DR with Ketanserin	K_e [nM] Antagonist	ED_{50} [μM] Agonist	DR with ICS 205-930	K_e [nM] Antagonist
Serotonin	0.447 ± 0.26 (11)			0.64 ± 0.25[d] (6)		
2-Me-Serotonin				2.95 ± 1.29 (15)		
Spiperone			0.38 ± 0.11[e] (6)			
YM-09151-2			17.87 ± 5.57 (8)			254.79 ± 58.61[b] (4)

[b]There was a maximum depression at each concentrations, studied.

[d]Determined in the presence of 100 nM ketanserin.

[e]EDTA was used in the experiment instead of ascorbic acid.

Table 5

AFFINITIES OF STANDARD COMPOUNDS AT μ/δ/κ-OPIOID RECEPTORS OF GUINEA-PIG BRAIN MEMBRANES AND HUMAN RECEPTORS ON CHO CELLS

	K_i (nM)					
Cold Ligand	μ in μ-CHO [^{3}H]DAMGO	μ in GP Brain [^{3}H]DAMGO	δ in δ-CHO [^{3}H]DPDPE-Cl	δ in GP Brain [^{3}H]DPDPE-Cl	κ_1 in κ-CHO [^{3}H]U69,593	κ_1 in GP Brain [^{3}H]U69,593
DAMGO	0.5 ± 0.05	1.1 ± 0.2	300.0 ± 58.6	180.4 ± 16	305.5 ± 46	1,841 ± 22
Morphine - sulfate	1.1 ± 0.05	2.0 ± 0.3	140.0 ± 1.5	50.0 ± 0.6	46.9 ± 14.5	33.9 ± 9
Normorphine-$HClO_4$	1.7 ± 0.25	3.9 ± 0.03	85.5 ± 1.0	60.5 ± 0.3	16.3 ± 2.2	64.5 ± 14
Fentanyl - HCl	0.7 ± 0.3	1.0 ± 0.1	152.7 ± 38.3	73.8 ± 3.5	84.8 ± 19.4	151.2 ± 5.2
Etonitazene	0.2 ± 0.1	1.6 ± 0.15	184.6 ± 121	141.9 ± 1.3	116.3 ± 11.7	595.0 ± 9.2
PL017	7.0 ± 1.0	8.1 ± 0.1	>10,000	>10,000	>10,000	>10,000
Dihydromorphine	1.7 ± 0.4	0.8 ± 0.2	203.4 ± 67.2	46.7 ± 4.9	83.8 ± 6.7	79.0 ± 8.7
Codeine Sulfate	135.2 ± 10.7	152.0 ± 33	>10,000	>10,000	-	>10,000
(-)Methadone - HCl	0.6 ± 0.2	1.4 ± 0.05	132.2 ± 10.7	37.3 ± 2.3	323.5 ± 18.3	728.0 ± 120
Nalmefene - HCl	0.3 ± 0.15	0.3 ± 0.08	7.3 ± 3.6	2.6 ± 0.1	0.3 ± 0.15	0.3 ± 0.1
Levorphanol - tartarate	0.3 ± 0	0.3 ± 0.02	14.7 ± 3.2	4.2 ± 0.3	1.5 ± 0.25	3.4 ± 0.7
Diprenorphine - HCl	0.8 ± 0.05	0.2 ± 0.06	0.5 ± 0.1	0.3 ± 0.05	0.2 ± 0.05	0.4 ± 0.2
Buprenorphine	1.5 ± 0.8	1.3 ± 0.15	4.5 ± 0.4	1.6 ± 0.07	0.8 ± 0.05	1.5 ± 0.25
CTAP - NH_2	2.3 ± 0.65	0.5 ± 0.05	365 ± 82	6.5 ± 1.3	>10,000	1,054 ± 4

Table 5 (continued)

AFFINITIES OF STANDARD COMPOUNDS AT μ/δ/κ-OPIOID RECEPTORS OF GUINEA-PIG BRAIN MEMBRANES AND HUMAN RECEPTORS ON CHO CELLS

	K_i (nM)					
Cold Ligand	μ in μ-CHO [^{3}H]DAMGO	μ in GP Brain [^{3}H]DAMGO	δ in δ-CHO [^{3}H]DPDPE-Cl	δ in GP Brain [^{3}H]DPDPE-Cl	κ_1 in κ-CHO [^{3}H]U69,593	κ_1 in GP Brain [^{3}H]U69,593
(-)Naloxone - HCl	1.4 ± 0.05	1.5 ± 0.02	67.5 ± 40	19.8 ± 0.7	2.5 ± 0.3	3.8 ± 0.9
Naltrexone - HCl	0.2 ± 0	0.4 ± 0.05	10.8 ± 3.0	6.5 ± 1.3	0.4 ± 0.1	0.6 ± 0.1
β-FNA - HCl	0.3 ± 0.05	0.4 ± 0.05	12.8 ± 0.95	7.7 ± 2.4	0.2 ± 0	0.9 ± 0.05
TIPPΨ	>10,000	>10,000	1.0 ± 0.7	0.6 ± 0.1	>10,000	>10,000
DPDPE - Cl	-	180.0 ± 1.2	0.3 ± 0.05	0.3 ± 0.1	-	>10,000
DPDPE - OH	503.6 ± 10.0	>10,000	1.7 ± 0.1	2.8 ± 0.04	>10,000	>10,000
DSLET - OH	6.9 ± 0.7	20.6 ± 3.6	0.5 ± 0.1	0.5 ± 0.1	>10,000	>10,000
DADLE - OH	1.8 ± 0.25	3.2 ± 0.05	0.7 ± 0.1	0.3 ± 0.01	>10,000	>10,000
Deltorphin-II	2,082 ± 998		1.5 ± 0.1		>10,000	>10,000
Leu-Enkephalin	7.4 ± 0.45	21.7 ± 1.4	2.1 ± 0.4	1.6 ± 0.5	>10,000	>10,000
β-Endorphin - OH	1.6 ± 0.1	2.3 ± 0.5	5.4 ± 0.45	1.6 ± 0.45	11.4 ± 1.2	43.5 ± 4.1
Naltrindole	6.3 ± 2.3	0.2 ± 0.01	0.2 ± 0.05	0.09 ± 0	10.1 ± 0.65	7.8 ± 0.1
BNTX	1.7 ± 0.05	1.9 ± 0.5	3.7 ± 2.5	4.2 ± 0.1	3.7 ± 1.35	7.1 ± 1.7
NTB	12.5 ± 2.1	6.5 ± 1.1	0.1 ± 0.04	0.06 ± 0	4.1 ± 0.7	10.2 ± 2.4
U69,593	1,145 ± 335	692.0 ± 97	>10,000	1,358± 118	0.3 ± 0	0.7 ± 0.05

Table 5 (continued)

AFFINITIES OF STANDARD COMPOUNDS AT μ/δ/κ-OPIOID RECEPTORS OF GUINEA-PIG BRAIN MEMBRANES AND HUMAN RECEPTORS ON CHO CELLS

	K_i (nM)					
Cold Ligand	μ in μ-CHO [^{3}H]DAMGO	μ in GP Brain [^{3}H]DAMGO	δ in δ-CHO [^{3}H]DPDPE-Cl	δ in GP Brain [^{3}H]DPDPE-Cl	κ_1 in κ-CHO [^{3}H]U69,593	κ_1 in GP Brain [^{3}H]U69,593
U50,488H	290.0 ± 14.3	294.0 ± 49	>10,000	>10,000	0.2 ± 0.05	0.2 ± 0.05
(-)EKC	0.3 ± 0.15	0.4 ± 0.04	3.4 ± 0	2.0 ± 0.07	0.1 ± 0.03	0.1 ± 0.01
(-)Bremazocine	0.2 ± 0.04	0.1 ± 0	0.9 ± 0.5	0.3 ± 0.07	0.03 ± 0.00	0.1 ± 0.03
Etorphine - HCl	0.3 ± 0.05	1.5 ± 0.35	1.5 ± 0.6	0.7 ± 0.07	0.2 ± 0.05	0.8 ± 0.20
Nalorphine - HCl	1.2 ± 0.2	1.9 ± 0.25	44.5 ± 2.9	19.3 ± 4.3	0.8 ± 0.05	1.7 ± 0.1
Nor-BNI $(HCl)_2$	21.0 ± 5	8.3 ± 1.2	5.7 ± 0.9	6.3 ± 0.4	0.2 ± 0.05	0.3 ± 0.10
Dynorphin (1-8)-OH	4.0 ± 0.9		3.6 ± 0.05	1.5 ± 0.7	0.2 ± 0.1	1.4 ± 0.4
Dynorphin (1-11) - OH	1.5 ± 0.5	2.3 ± 0.5	10.4 ± 0	1.8 ± 0.2	0.2 ± 0.05	0.1 ± 0
Dynorphin (1-13) - OH	4.5 ± 0.1	3.3 ± 0.1	14.3 ± 0.8	16.3 ± 0.9	0.5 ± 0.05	0.4 ± 0.1
Dynorphin (1-17) - OH	7.7 ± 2.2	8.1 ± 0.2	42.7 ± 8.6	5.8 ± 0.8	1.7 ± 0.85	1.7 ± 0
Dynorphin B - OH	3.0 ± 0.6	5.5 ± 0.2	14.7 ± 5.1	4.2 ± 0.25	0.3 ± 0.05	0.9 ± 0.1
(-) Cyclazocine	0.1 ± 0	0.1 ± 0.0	0.8 ± 0.05	0.6 ± 0.05	0.1 ± 0	0.1 ± 0.02
(-) Pentazocine	3.9 ± 0.7	5.7 ± 0.9	49.3 ± 15.1	32.7 ± 3.15	2.2 ± 0.2	4.4 ± 0.1
NalBzoH	1.8 ± 0.45	0.2 ± 0	6.0 ± 1.4	1.4 ± 0.13	0.3 ± 0.1	0.4 ± 0.1
(-) WIN 44,441	0.1 ± 0	0.1 ± 0.03	1.4 ± 0.5	0.9 ± 0.07	0.2 ± 0.1	0.2 ± 0

Table 6

RESULTS OF STANDARD COMPOUNDS IN THE OPIATE BIOASSAYS

	Guinea Pig Ileum							Mouse Vas Deferens			
Compound	IC_{50} (nM)	DR with CTAP	K_e of CTAP	Ratio	DR with Nor-BNI	K_e of Nor-BNI	Ratio	IC_{50} (nM)	DR with Naltrindole	K_e of Naltrindole	Ratio
DAMGO	8.25 ± 2.0 (13)	4.98 ± 0.3 (4)	25.31 ± 2.54 (4)	1.000	1.74 ± 0.14 (6)	27.67 ± 4.52 (6)	0.002	177.60 ± 134 (7)	0.93 ± 0.10 (4)	N.D.	
Morphine	24.75 ± 2.4 (4)	4.27 ± 0.18 (2)	30.67 ± 1.66 (2)	0.707	1.05 ± 0.06 (2)	222.22 (1)	0.003	2,131 ± 904 (4)	0.92 ± 0.38 (4)	N.D.	
Normorphine	47.30 ± 13 (15)	3.02 ± 0.28 (4)	50.25 ± 6.6 (4)	0.500	1.90 ± 0.39 (4)	26.47 ± 13.6 (4)	0.002	511.9 ± 51 (4)	1.06 ± 0.07 (4)	8.38 ± 0 (2)	0.0025
Dihydromorphine	42.39 ± 6.61 (3)	12.46 ± 0.15 (2)	17.46 ± 0.23 (2)	1.354	1.12 ± 0.18 (2)			8,113 ± 2,729 (3)	2.67 ± 0.82 (2)		
Fentanyl	1.86 ± 0.64 (8)	3.12 ± 0.82 (4)	53.86 ± 23.10 (4)	0.403	1.18 ± 0.09 (2)	132.6 ± 69.6 (2)	0.001	18.07 ± 3.0 (3)	0.87 ± 0.07 (3)	N.D.	
Etonitazene	0.89 ± 0.16 (4)	10.38 ± 0.67 (2)	10.69 ± 0.76 (2)	2.028	1.70 ± 0.27 (2)	30.84 ± 11.84 (2)	0.002	1.85 ± 0.07 (2)	0.82 ± 0.11 (2)	N.D.	
A-PL017	18.11 ± 2.83 (4)	7.19 ± 0.74 (2)	16.29 ± 1.96 (2)	1.331	1.49 ± 0.15 (2)	43.27 ± 13.2 (2)	0.001	240.5 ± 63 (2)	0.89 ± 0.11 (4)	N.D.	
(-)Methadone	45.83 ± 3.6 (3)	1.19 ± 0.14 (4)	408 ± 100 (3)	0.062	1.20 ± 0.06 (2)	108.3 ± 35.4 (2)	0.001	452.5 ± 251 (2)	0.94 ± 0.09 (2)	N.D.	

Oxycodone	**323.80 ± 116 (4)**	**2.30 ± 0.25 (2)**	**78.66 ± 15 (2)**	**0.322**	**1.45 ± 0.10 (3)**	**45.84 ± 11.5 (3)**	**0.001**	**6,330 ± 3,140 (3)**	**0.79 ± 0.08 (2)**	**N.D.**	

Table 6(continued)

RESULTS OF STANDARD COMPOUNDS IN THE OPIATE BIOASSAYS

	Guinea Pig Ileum							Mouse Vas Deferens			
Compound	IC_{50} (nM)	DR with CTAP	K_e of CTAP	Ratio	DR with Nor-BNI	K_e of Nor-BNI	Ratio	IC_{50} (nM)	DR with Naltrindole	K_e of Naltrindole	Ratio
Nalorphine	29.20 ± 15[a] (9)	0.90 ± 0.14 (2)	N.D.		643.9 ± 83.5 (2)	0.03 ± 0.004 (2)	2.000	a			
β-Endorphin	59.43 ± 6.26 (4)	5.86 ± 0.79 (2)	20.87 ± 3.37 (2)	0.928	4.24 ± 1.55 (2)	6.98 ± 3.34 (2)	0.007	67.99 ± 19.0 (3)	7.98 ± 2.3 (4)	0.156 ± 0.05 (4)	0.147
NalBzoH	b				b			b			
Nalmefene	c				c			c			
Naloxone	d				d			d			
Naltrexone	e				e			e			
CTAP	f										
Buprenorphine	8.13 ± 3.55[g] (4)							21.39 ± 14.3 (3)			

[a]Nalorphine is a κ-opioid receptor agonist and a μ/δ receptor antagonist. In the GPI the μ antagonist activity was determined in the presence of 20 nM Nor-BNI. Its pA_2 value at μ is 7.49/-0.87, and at δ is 6.79/-1.05.
[b]NalBzoH is an antagonist at all three opioid receptors. The pA_2 value at μ is 8.81/-1.02, at δ is 7.76/-0.96, and at κ is 7.76/-1.19. [c]Nalmefene is an antagonist at all three opioid receptors. The pA_2 value at μ is 9.38/-1.05, at δ is 7.82/-1.15, and at κ is 8.48/-1.01. [d]Naloxone is an antagonist at all three opioid receptors. The pA_2 value at μ is 8.51/-1.07, at δ is 7.30/-1.05, and at κ is 7.73/-0.99. [e]Naltrexone is an antagonist at all three opioid receptors. The pA_2 value at μ is 9.19/-1.08, at δ is 8.08/-1.09, and at κ is 8.11/-1.03. [f]CTAP is a very selective μ receptor antagonist. Its pA_2 value is 7.65 and the slope is -1.02. [g]The agonist activity could not be reversed neither with CTAP nor with nor-BNI. In β-FNA treated GPI the compound is a κ antagonist. The pA_2 value at the κ is 9.16/-1.28.

Table 6 (continued)

RESULTS OF STANDARD COMPOUNDS IN THE OPIATE BIOASSAYS

	Guinea Pig Ileum							Mouse Vas Deferens			
Compound	IC_{50} (nM)	DR with CTAP	K_e of CTAP	Ratio	DR with Nor-BNI	K_e of Nor-BNI	Ratio	IC_{50} (nM)	DR with Naltrindole	K_e of Naltrindole	Ratio
DPDPE	4,130 ± 870 (6)	5.84 ± 2.6 (3)	25.83 ± 15.3 (3)	0.980	1.54 ± 0.31 (4)	50.68 ± 35.1 (4)	0.001	4.11 ± 1.32 (80)	53.11 ± 17.6 (8)	0.021 ± 0.007 (8)	1.000
DSLET	59.30 ± 3.78 (4)	5.36 ± 1.35 (4)	24.50 ± 6.9 (4)	1.030	1.72 ± 0.13 (4)	28.55 ± 5.6 (4)	0.002	1.23 ± 0.40 (11)	46.84 ± 8.0 (3)	0.022 ± 0.004 (3)	0.955
DTLET	41.70 ± 14.52 (4)	5.33 ± 0.04 (2)	23.12 ± 0.19 (2)	0.938	1.57 ± 0.03 (2)	35.13 ± 1.74 (2)	0.002	0.32 ± 0.14 (4)	31.25 ± 6.7 (4)	0.034 ± 0.007 (4)	0.612
DADLE	13.39 ± 7.4 (3)	4.33 ± 0.60 (2)	30.58 ± 5.5 (2)	0.709	1.53 ± 0.02 (2)	38.13 ± 1.54 (2)	0.002	1.60 ± 0.30 (4)	16.22 ± 4.01 (3)	0.069 ± 0.016 (3)	0.304
Leu - Enkephalin*	87.35 ± 9.90 (4)	4.63 ± 1.26 (2)	29.38 ± 10.26 (2)	0.659	1.94 ± 0.04 (2)	21.41 ± 0.81 (2)	0.002	7.38 ± 2.40 (6)	28.07 ± 4.45 (6)	0.038 ± 0.001 (6)	0.577
Met - Enkephalin*	27.44 ± 3.75 (4)	5.08 ± 0.17 (2)	24.50 ± 1.06 (2)	0.791	6.07 ± 0.26 (2)	3.95 ± 0.20 (2)	0.012	1.52 ± 0.26 (3)	13.63 ± 2.74 (4)	0.082 ± 0.016 (4)	0.305
Dynorphin (1-9)*	4.69 ± 2.34 (4)	1.13 ± 0.20 (2)	357.14 (1)	0.054	75.48 ± 26.20 (2)	0.29 ± 0.10 (2)	0.161	12.22 ± 2.59 (4)	5.71 ± 2.90 (4)	0.281 ± 0.154 (4)	0.082
Naltrindole	h				h			h			
NTB					i			i			

*Experiments with dynorphins, Met- and Leu-enkephalins were done in the presence of enzyme inhibitors.

[h]**Naltrindole is an antagonist at all three opioid receptors. The pA_2 value at μ is 7.53/-1.13, at δ is 10.92/-0.83, and at κ is 7.61/-0.85.**

[i]**NTB is an antagonist at all three opioid receptors. The pA_2 value at μ is 7.95/-0.94, at δ is 10.55/-1.03, and at κ is 7.22/-1.02.**

Table 6 (continued)

RESULTS OF STANDARD COMPOUNDS IN THE OPIATE BIOASSAYS

	Guinea Pig Ileum							Mouse Vas Deferens			
Compound	IC_{50}(nM)	DR with CTAP	K_e of CTAP	Ratio	DR with Nor-BNI	K_e of Nor-BNI	Ratio	IC_{50} (nM)	DR with Naltrindole	K_e of Naltrindole	Ratio
BNTX	j				j			j			
TIPPΨ								k			
U 69,593	1.66 ± 0.63 (12)	0.68 ± 0.11 (4)	N.D.		363.0 ± 97.0 (7)	0.06 ± 0.017 (8)	1.000	208.30 ± 139 (8)	0.40 ± 0.10 (4)	N.D.	
U 50,488H	1.57 ± 0.50 (6)	0.52 ± 0.06 (2)	N.D.		430.1 ± 85.7 (4)	0.05 ± 0.01 (4)	1.250	94.33 ± 16.2 (3)	0.94 ± 0.20 (2)	N.D.	
(-)Bremazocine	0.067 ± 0.015 (4)	0.83 ± 0.12 (2)	N.D.		226.3 ± 34.3 (2)	0.09 ± 0.014 (2)	0.445	l			
Etorphine	0.055 ± 0.016 (4)	1.54 ± 0.04 (2)	185.76 ± 14.6 (2)	0.104	2.34 ± 0.18 (2)	15.03 ± 2.02 (2)	0.003	1.39 ± 0.20 (4)	0.82 ± 0.11 (4)	N.D.	
(±)EKC	0.44 ± 0.14 (8)	0.60 ± 0.09 (4)	N.D.		61.20 ± 6.8 (8)	0.36 ± 0.11 (8)	0.170	18.98 ± 7.0 (8)	0.88 ± 0.09 (4)	N.D.	
(-)EKC	0.15 ± 0.01 (4)	0.62 ± 0.01 (2)	N.D.		35.09 ± 8.47 (2)	0.61 ± 0.15 (2)	0.076	5.31 ± 1.89 (4)	0.90 ± 0.15 (4)	N.D.	
CI -977	0.15 ± 0.06 (6)	0.64 ± 0.10 (2)	N.D.		275.90 ± 25.0 (3)	0.07 ± 0.06 (3)	0.630	3.71 ± 2.40 (4)	0.89 ± 0.10 (4)	N.D.	

[j]BNTX is an antagonist at all three opioid receptors. The pA_2 value at μ is 8.56/-0.93, at δ is 8.90/-1.01, and at κ is 7.43/-0.78.

[k]TIPPΨ is a very selective, competitive δ-opioid receptor antagonist. The pA_2 value at δ is 9.17/-0.99.

[l]IC_{50} could not be determined. Very shallow dose-response curve.

Table 6 (continued)

RESULTS OF STANDARD COMPOUNDS IN THE OPIATE BIOASSAYS

	Guinea Pig Ileum							Mouse Vas Deferens			
Compound	IC_{50} (nM)	DR with CTAP	K_e of CTAP	Ratio	DR with Nor-BNI	K_e of Nor-BNI	Ratio	IC_{50} (nM)	DR with Naltrindole	K_e of Naltrindole	Ratio
CI -977	0.15 ± 0.06 (6)	0.64 ± 0.10 (2)	N.D.		275.90 ± 25.0 (3)	0.07 ± 0.06 (3)	0.630	3.71 ± 2.40 (4)	0.89 ± 0.10 (4)	N.D.	
Dynorphin (1-8)	71.76 ± 45.5 (7)	0.98 ± 0.17 (2)	N.D.		18.44 ± 4.53 (4)	1.21 ± 0.34 (4)	0.050	56.40 ± 5.0 (3)	2.77 ± 0.68 (3)	0.64 ± 0.29 (3)	0.033
Dynorphin (1-11)	1.03 ± 0.40 (4)	0.34 ± 0.02 (2)	N.D.		116.49 ± 21.2 (4)	0.18 ± 0.03 (2)	0.261	368.92 ± 56.0 (4)	0.75 ± 0.12 (4)	N.D.	
Dynorphin (1-13)OH	0.17 ± 0.07 (4)	1.05 ± 0.57 (2)	222.22 (1)	0.098	425.30 ± 40.4 (3)	0.05 ± 0.01 (4)	0.979	5.28 ± 2.2 (4)	0.89 ± 0.33 (4)	N.D.	
Dynorphin (1-13) NH_2	0.38 ± 0.18 (4)	1.33 ± 0.17 (2)	349.21 ± 179 (2)	0.056	125.81 ± 14.3 (2)	0.16 ± 0.02 (2)	0.286	7.02 ± 2.44 (4)	0.99 ± 0.09 (4)	N.D.	
Dynorphin A (1-17)	0.95 ± 0.08 (2)	1.52 ± 0.21 (2)	209.89 ± 86 (2)	0.121	102.20 ± 11.0 (2)	0.20 ± 0.02 (2)	0.302	29.30 ± 24.9 (2)	1.15 ± 0.05 (2)	7.32 ± 2.5 (2)	0.003
Dynorphin B	4.40 ± 1.54 (4)	1.08 ± 0.30 (2)	344.83 (1)	0.056	75.48 ± 26.2 (2)	0.28 ± 0.10 (2)	0.161	39.14 ± 7.39 (4)	2.10 ± 0.30 (4)	0.95 ± 0.21 (4)	0.024
Nor-BNI	m				m			m			

[m] Nor-BNI is a selective κ_1 antagonist. Its pA_2 value at the κ_1 receptor is 10.02 and the slope is -1.14. The pA_2 value at μ receptor is 7.26/-1.19, and at δ receptor is 7.87/-1.04.

Table 6 (concluded)

RESULTS OF STANDARD COMPOUNDS IN THE OPIATE BIOASSAYS

	Guinea Pig Ileum							Mouse Vas Deferens			
Compound	IC_{50} (nM)	DR with CTAP	K_e of CTAP	Ratio	DR with Nor-BNI	K_e of Nor-BNI	Ratio	IC_{50} (nM)	DR with Naltrindole	K_e of Naltrindole	Ratio
(-)SKF10,047	10.50 ± 3.9[n] (7)	0.44 ± 0.02 (2)	N.D.		273.10 ± 47 (2)	0.08 ± 0.01 (2)	0.800	n			
(-)Pentazocine	170.30 ± 69.2 (4)	0.73 ± 0.38 (2)	N.D.		6.10 ± 1.00 (2)	4.00 ± 0.8 (2)	0.015	o			
(-)Cyclazocine	1.05 ± 0.4 (4)	1.00 ± 0.00 (2)	N.D.		33.39 ± 25.2 (2)	0.89 ± 0.69 (2)	0.067	p			

[n] In the GPI the μ antagonist activity was determined in the presence of 20 nM Nor-BNI.The pA_2 value in the GPI is 7.69/-1.22. In the MVD from 10^{-9} to 10^{-6} M slight inhibition; from 5×10^{-6} to 5×10^{-5} M enhancement.The pA_2 value in the MVD at the μ receptor is 8.20/-1.20, and at the δ is 7.55/-0.99.

[o] IC_{50} could not be determined.

[p] IC_{50} could not be determined from 10^{-9} to 5×10^{-5} M (maximum inhibition = 17%).The pA_2 value in the MVD at the μ receptor is 9.02/-1.01, and at the δ is 8.23/-0.95.The experiments were done in the presence of 5 nM nor-BNI to block any agonist effect on the tissue.

Table 7

STIMULATION OF [^{35}S]GTPγS BINDING TO μ, δ, AND κ RECEPTORS

	Human μ-CHO Cell Membranes		Human δ-CHO Cell Membranes		Human κ-CHO Cell Membranes	
Cold Ligand	EC_{50} (nM)	% Stimulation	EC_{50} (nM)	% Stimulation	EC_{50} (nM)	% Stimulation
DAMGO	13.7 ± 5.28	100	flat		4,365 ± 1,661	62 ± 21
Morphine	15.6 ± 0.5	93 ± 2.8	316.5 ± 4.9	103 ± 7	484 ± 213	62 ± 7
Normorphine	47.4 ± 11	114 ±11	418.0 ± 48.8	96 ± 24	1,443 ± 504	86 ± 3
Fentanyl	8.1 ± 0.4	100 ± 12	515.0 ± 102	86 ± 19	2,368 ± 534	30 ± 4
Etonitazene	1.0 ± 0	119 ± 19	300.5 ± 96	114 ± 23	7,312 ± 3,774	24 ± 1
PL017	97.5 ± 21.9	109 ± 22	flat			
Dihydromorphine	35.9 ± 15	109 ± 5	225 ± 128	106 ± 4	1,015 ± 347	47 ± 3
(-)Methadone	26.6 ± 14.3	116 ± 20	980 ± 99	106 ± 21	4,943 ± 400	25 ± 16
Nalmefene	flat		30.5 ± 20	66.5 ± 13	flat	
Diprenorphine	flat		0.8 ± 0.2	98.5 ± 3.5	0.26 ± 0.06	47 ± 4
Buprenorphine	2.3 ± 1.7	66 ± 36	flat		flat	
CTAP	flat		flat		flat	
(-)Naloxone	flat		flat		flat	

% Stimulation compared to DAMGO, DPDPE or U69,593, respectively.

Table 7 (continued)

RESULTS ON HUMAN μ/δ/κ-CHO CELL MEMBRANES USING GTPγS BINDING

	Human μ-CHO Cell Membranes		Human δ-CHO Cell Membranes		Human κ-CHO Cell Membranes	
Cold Ligand	EC_{50} (nM)	% Stimulation	EC_{50} (nM)	% Stimulation	EC_{50} (nM)	% Stimulation
Naltrexone	flat		flat		flat	
β-FNA	3.1 ± 1.3	19 ± 0	flat		5.1 ± 1.4	78 ± 9
β-CNA	flat		flat		3.9 ± 1.2	52 ± 22
DPDPE	flat		1.3 ± 0.5	100	flat	
DSLET	74.6 ±15	134 ± 65	0.7 ± 0.4	116 ± 22	flat	
DADLE	14.0 ± 2.8	89 ± 15	0.6 ± 0.12	107 ± 9	12,780 ± 6,300	69 ± 9
Deltorphin-II	flat		0.35 ±0.07	134 ± 16		
Leu-Enkephalin	25.5 ± 0.8	11 ± 4	1.35 ± 0.2	104 ± 21	4,160 ± 1,683	89 ± 15
β-Endorphin	24.5 ± 3.5	116 ± 17	13.6 ± 11.8	82.5 ± 11	971 ± 175	77
Naltrindole	flat		flat		flat	
NTB	flat		flat		flat	
U69,593	flat		flat		26.15 ± 10.7	100
U50,488H	flat		flat		9.31 ± 2.54	93 ± 11
(-)EKC	1.3 ± 0.4	146 ± 85	2.3 ± 1.3	99 ± 1	0.41 ± 0.14	88 ± 11

% Stimulation compared to DAMGO, DPDPE or U69,593, respectively.

Table 7 (continued)

RESULTS ON HUMAN μ/δ/κ-CHO CELL MEMBRANES USING GTPγS BINDING

	Human μ-CHO Cell Membranes		Human δ-CHO Cell Membranes		Human κ-CHO Cell Membranes	
Cold Ligand	EC_{50} (nM)	% Stimulation	EC_{50} (nM)	% Stimulation	EC_{50} (nM)	% Stimulation
(-)Bremazocine	flat		3.8 ± 1.7	101 ± 8	0.053 ± 0.008	84 ± 13
Etorphine	0.66± 0.06	117 ± 24	1.5 ± 0.2	107 ± 2	2.02 ± 0.57	95 ± 16
Nalorphine	flat		59.7 ± 22	58 ± 15	2.56 ± 0.06	14 ± 0
Nor-BNI	flat		flat		flat	
Dynorphin (1-8)	58.5 ± 27.6	105 ± 9	3.4 ± 0.7	76 ± 22	116 ± 37	94 ± 4
Dynorphin (1-9)	24.0 ± 0	96 ± 0.7	6.6 ± 1.3	84 ± 5	9.50 ± 6.0	66
Dynorphin (1-11)	43.0 ± 2.8	95 ± 2.8	39.2 ± 1.3	85 ± 5	1.43 ± 0.66	92 ± 4
Dynorphin (1-13)	68.5 ± 4.9	115	81.4 ± 10.4	120	2.46 ± 0.93	93 ± 10
Dynorphin (1-17)	65.0 ± 34	99 ± 1.4	72.0 ± 12	99 ± 3	5.65 ± 2.08	97 ± 5
Dynorphin B	63.5 ± 3.5	112 ± 23	54.8 ± 0.8	97	5.80 ± 1.4	82
(-) Cyclazocine	1.2 ± 0.07	33 ± 18	2.9 ± 1.9	82 ± 9	0.80 ± 0.2	80
(-) SKF 10,047	flat		9.4 ± 2.3	70 ± 9	5.38 ± 2.3	49 ± 13
(-) Pentazocine	36.0 ± 7.1	35 ± 4	148.5 ± 40.3	85 ± 10	27.5 ± 7.8	39 ± 14
NalBzoH	flat				6.14 ± 4.19	42 ± 6

% Stimulation compared to DAMGO, DPDPE or U69,593, respectively.