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EVALUATION OF A SERIES OF *N*-ALKYL BENZOMORPHANS IN CELL LINES EXPRESSING TRANSFECTED δ- AND μ-OPIOID RECEPTORS

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Abstract—Transfection of individual opioid receptors in Chinese hamster ovary (CHO) cells provides a pure, homogeneous population of receptors for screening drug candidates, and an alternative to the use of selective ligands. To evaluate the potential of this system, we chose a series of (−)-5,9 α-dimethyl-2-hydroxy-*N*-substituted-6,7-benzomorphans, for which the receptor selectivity and *in vivo* activity had been characterized recently, and tested them in CHO cells stably transfected with either the rat δ-opioid receptor or the mouse μ-opioid receptor. [^{3}H]Diprenorphine was used to measure opioid receptors in P_2 membrane preparations. A B_{max} of 7.58 ± 0.8 pmol/mg protein and a K_d of 0.42 ± 0.04 nM was obtained in the μ-opioid receptor expressing cell line used in these studies. In addition, [^{3}H]naltrindole was used to confirm the δ-specificity of the cloned receptor. Both compounds gave a B_{max} of 1.2 pmol/mg in the CHO cells expressing the rat δ-opioid receptor. Displacement assays were performed with eleven (−)-*N*-alkyl-benzomorphans in the absence and presence of 150 mM NaCl, as well as known δ- and μ-selective agonists. Sodium reduced agonist affinity in the transfected cell lines. The benzomorphan compounds displayed a range of affinities in the μ- and δ-opioid receptor expressing cell lines. Good correlations were found between their affinities at the cloned μ- and δ-opioid receptors and those in rat brain and monkey cortex (r^2 from 0.73 to 0.89, $P < 0.001$). Comparative analysis of K_i values with *in vivo* potency in the mouse tail flick test indicated a high degree of correlation between antinociception and affinity in the μ-opioid receptor cell line ($r^2 = 0.83$, $p < 0.0001$). Lesser correlations were found between antinociception in the mouse and affinity at the rat μ-opioid receptor ($r^2 = 0.6610$) and at the monkey μ-opioid receptor ($r^2 = 0.695$). In sum, these studies indicate that the cell lines expressing the cloned μ- and δ-opioid receptors are appropriate models for determining the binding affinities of this class of opioid compounds. The diminishing correlations found between species when comparing *in vitro* and *in vivo* activity suggest that caution should be taken when extrapolating binding data to pharmacological activity among species.

Key words: opioid receptor; CHO cell line; radioligand binding; sodium

A series of *N*-alkyl-benzomorphans has been synthesized and previously evaluated for *in vivo* and *in vitro* activity [1]. The pharmacological activities for these compounds include antinociception, with the (−)-methyl, -pentyl, -heptyl and -hexyl compounds equipotent to morphine in tail flick, phenylquinone and hot plate assays; and narcotic antagonism with the (−)-propyl compound. Binding assays with selective opioid ligands revealed activity at μ, κ, δ and σ receptors. All five of these compounds were potent at the μ receptor in both rat and monkey preparations (<0.05 μM), and at the κ receptor (<0.1 μM), and the propyl, pentyl and heptyl compounds showed reasonable affinity for the δ receptor (<0.1 μM).

Several groups have reported the molecular cloning of μ-, δ- and κ-opioid receptors [2–20]. Transfection of individual opioid receptors into CHO† cells can now be used to provide a pure, homogeneous population for screening drug candidates. Instead of using selective ligands, we can use specific receptors, with the caveat that the receptors are not in their "native" environment. To evaluate the feasibility of the CHO expression system, we chose a series of opioid-active compounds for which the pharmacology and receptor selectivity had been defined recently. Stably transfected CHO cell lines expressing either the mouse μ-opioid receptor or the rat δ-opioid receptor [10] were used in these studies. CHO cells stably transfected with μ- or δ-opioid receptors displayed affinities for *N*-alkyl-benzomorphans that were similar, yet distinct, from those obtained using selective opioid ligands in brain homogenates.

MATERIALS AND METHODS

Cell culture

A clonal CHO cell line, rdor4, was made using standard techniques [10]. A clonal CHO cell line, mMOR, expressing the mouse μ-opioid receptor was provided by Duane Keith, Daniel Kaufman, Tuyet Tran and Chris Evans, UCLA. Both cell lines were maintained in DMEM + 10% fetal bovine serum + 0.3 mg/mL G418 (LTI, Gaithersburg, MD).

Receptor binding

[^{3}H]Diprenorphine was used to measure opioid receptors in P_2 membrane preparations (36 Ci/mmol; Amer-

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† Abbreviations: CHO, Chinese hamster ovary; DPDPE, [D-Pen^{2,5}]-enkephalin; DAMGO [D-Ala^2,*N*-Me-Phe^4,Gly-ol^5]-enkephalin; mMOR, mouse μ-opioid receptor; and rDOR, rat δ-opioid receptor.

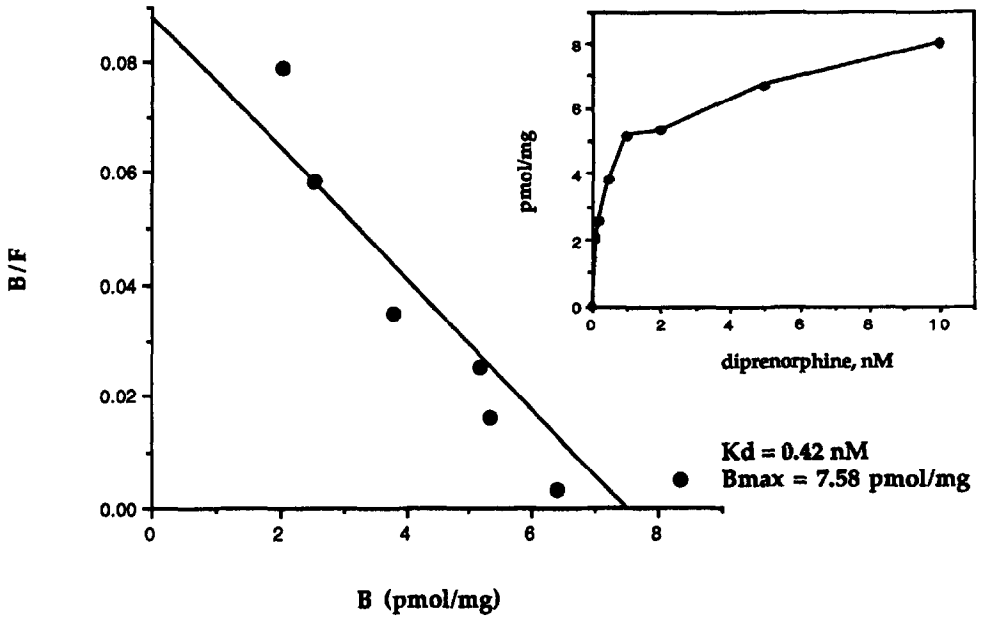


Fig. 1. Scatchard–Rosenthal and saturation (inset) analysis of [^{3}H]diprenorphine binding in CHO cells stably transfected with the mouse μ-opioid receptor (mMOR). These data are representative of three experiments performed in triplicate.

sham, Arlington Heights, IL). P_2 membranes were prepared using standard techniques [21]. Membranes (5–15 μg protein) were added to tubes containing [^{3}H]diprenorphine in 25 mM HEPES, pH 7.7, and incubated for 90 min at 24°. Solutions were titrated with KOH (and not NaOH). Binding was terminated by rapid filtration through Whatman GF/B filters in a cell harvester. Nonspecific binding was determined with 1 μM diprenorphine. Saturation assays were conducted with 7 concentrations of [^{3}H]diprenorphine ranging from 0.1 to 10 nM. Competition assays were performed using 1 nM [^{3}H]diprenorphine and six concentrations (0.1 nM to 10 μM) of displacing ligands, in the absence or presence of 150 mM NaCl. Assays were performed in triplicate, and results represent the combined data of two to seven independent experiments.

In addition, [^{3}H]naltrindole (NEN, 29 Ci/mmol) was used to evaluate δ-opioid receptor binding. The cells were harvested and homogenized in 50 mM Tris–HCl, pH 7.8, 5.0 mM $MgCl_2$, 1 mM EGTA, 10 μg/mL leupeptin, 10 μg/mL bacitracin, and 0.5 μg/mL aprotinin (buffer 1; [6]). The homogenate was centrifuged at 24,000 *g* for 7 min at 4°. The pellet was homogenized in buffer 1 and centrifuged at 48,000 *g* for 20 min at 4°. The pellet was homogenized in buffer 1 and used for the binding studies. Saturation studies were performed with six different concentrations of [^{3}H]naltrindole (0.01 to 3 nM) in assay buffer (50 mM Tris–HCl, 5.0 mM $MgCl_2$, 1 mg/mL BSA, 50 μg/mL bacitracin, 30 μM bestatin, 10 μM captopril and 0.1 mM phenylmethylsulfonyl flouride adjusted to pH 7.4) [22]. Membranes (15 μg) were added to this assay buffer and incubated for 3 hr at 25°. Nonspecific binding was determined using 10 μM naloxone. The final volume of the assay samples was 1.0 mL. The assay was terminated by filtration through Whatman GF/B filter strips previously treated with 0.5% polyethylenimine for more than 1 hr. The filtrates were washed three times with ice-cold normal saline.

Data analysis

The B_{max} and K_d values generated from Scatchard–Rosenthal analysis were obtained using the KELL package of binding analysis programs for the Macintosh computer (Biosoft, Milltown, NJ). The K_i values were generated from IC_{50} values of the displacement curves with the KELL package. Statistical analysis (linear regression and rank order correlations) were performed using the StatView 4.0 program for the Macintosh computer (Abacus Concepts, Berkeley, CA). Potencies of the benzomorphans as agonists or antagonists were weighed equally, and inactive compounds were assigned the cutoff dose of 30 mg/kg for the linear regression analysis. Compounds with K_i values > 1 μM and > 10 μM (in the presence of sodium) were assigned values of 1 μM and 10 μM, respectively, for data analysis.

RESULTS

These assays were performed in two separate cell lines. The mMOR or the rDOR was stably expressed in CHO lines. We found that the CHO cells lacked any opioid receptors prior to transfection, as has been demonstrated previously (data not shown; [23]). Diprenorphine was used as the radioligand in these assays. Although diprenorphine is a non-selective opioid antagonist, the cells are expressing individual cloned receptors, which are, by definition, a single subtype. In subsequent experiments, the selective δ antagonist, naltrindole, was evaluated in the δ receptor cell line. Naltrindole gave a B_{max} similar to that of diprenorphine (see Fig. 2B).

Specific binding was found to be linear at low protein concentrations for the transfected cell lines. In the mMOR and rDOR expressing cell lines, specific binding dropped off at protein concentrations over 15 μg/mL (data not shown). Thus, 5–15 μg/mL of membrane protein was used in subsequent assays.

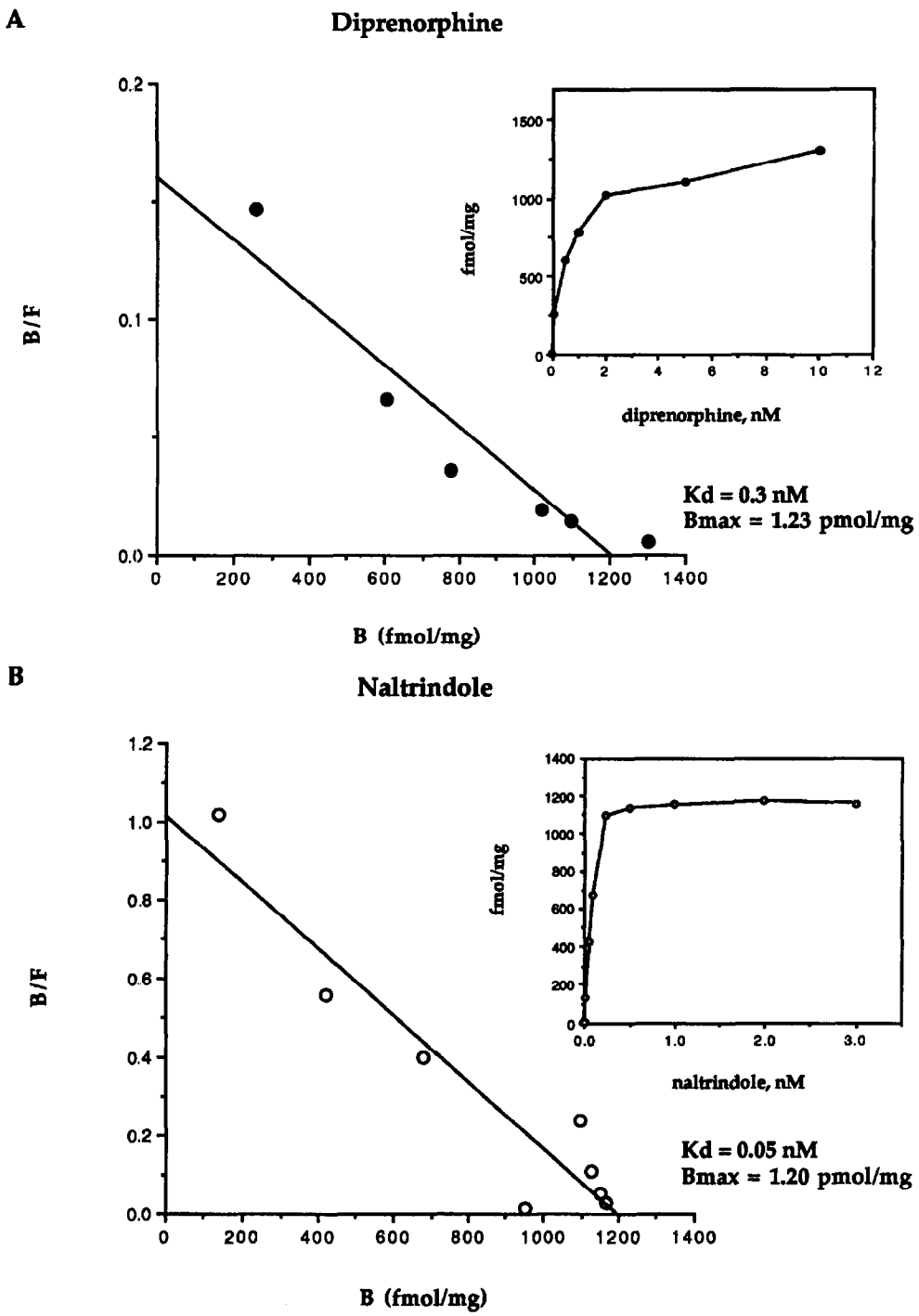
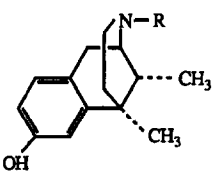


Fig. 2. Comparison of Scatchard–Rosenthal and saturation (inset) analysis of [^{3}H]diprenorphine (A) and [^{3}H]naltrindole (B) binding in CHO cells stably transfected with the rat δ-opioid receptor (rdor4). These data are representative of three experiments performed in triplicate.

Scatchard analysis of saturation isotherms with the transfected cell lines revealed saturable, high-affinity binding. Representative experiments are shown in Figs. 1 and 2. The mMOR cell line bound diprenorphine with a B_{max} of 7.58 ± 0.8 pmol/mg protein and a K_d of 0.42 ± 0.04 nM (Fig. 1). The rDOR cell line bound diprenorphine with a B_{max} of 1.16 ± 0.9 pmol/mg protein and a K_d of 0.51 ± 0.15 nM (see representative experiment in Fig. 2A). Diprenorphine binding to the cell lines was compatible with a single site (Hill coefficients of 0.98 ±



(-)- 5,9 α- dimethyl- 2'- hydroxy- N- substituted- 6,7- benzomorphans

Fig. 3. Structure of *N*-alkyl-substituted benzomorphans.

Table 1. Comparison of *in vitro* and *in vivo* activities of *N*-alkyl-substituted benzomorphans in transfected cells and animals

	R	mMOR K_i (nM)	mMOR + NaCl K_i (nM)	Monkey [1] K_i (nM)	Rat [1] K_i (nM)	Mouse tail flick [1] ED_{50} (mg/kg)		rDOR K_i (nM)	rDOR + NaCl K_i (nM)	Monkey [1] K_i (nM)
C0	H	95.97 ± 38	1,360.00	54.40	23.00	13% at 30		569 ± 45	1,010.00	449.00
C1	methyl	14.08 ± 2.4	72.00	4.20	11.00	0.80		126.8 ± 49	2,017.00	152.00
C2	ethyl	55.53 ± 14	69.00	16.30	32.00	3.7*		189.67 ± 35	234.00	158.00
C3	*n*-propyl	7.93 ± 1.7	12.50	1.50	7.60	0.4*		272.27 ± 96	94.80	37.10
C4	*n*-butyl	10.95 ± 4.2	24.90	3.40	13.00	1.5*		93.26 ± 39	87.00	77.70
C5	*n*-pentyl	38.99 ± 14	208.50	15.10	16.00	1.30		132.68 ± 33	252.00	98.00
C6	*n*-hexyl	8.28 ± 2.6	495.50	47.30	4.70	1.10		33.86 ± 15	540.00	66.50
C7	*n*-heptyl	27.83 ± 18.7	370.00	30.10	8.80	1.70		125.18 ± 44	>1,000	57.40
C8	*n*-octyl	67.05 ± 32.8	830.00	59.00	30.00	10.30		105.8 ± 33	>1,000	114.00
C9	*n*-nonyl	285.9 ± 75	>10,000	183.00	151.00	I†		330.67 ± 168	>10,000	464.00
C10	*n*-decyl	640.1 ± 330	>10,000	997.00	507.00	I		4,335 ± 110	>10,000	2,584.00
	Morphine	8.80				0.73	DPDPE	26.7 ± 2.3		
	DAMGO	2.02					Deltorphin	1.8 ± 2.2		

The mMOR and rDOR binding assays were performed using 1 nM [^{3}H]diprenorphine and 0.1 nM to 10 μM displacing ligands. The K_i values in the absence of sodium are means ± SEM (N = 3–7). The K_i values in the presence of 150 mM NaCl are averages of duplicate experiments. The rest of the values in the table are taken from published data [1]. Opioid K_i values for the μ-and δ-opioid receptor from monkey cortex and rat brain minus cerebellum were obtained in displacement studies using [^{3}H]DAMGO and [^{3}H]DPDPE, respectively [1].

* Compound acts as a morphine antagonist in the mouse tail flick assay.

† Compound was inactive in the mouse tail flick assay.

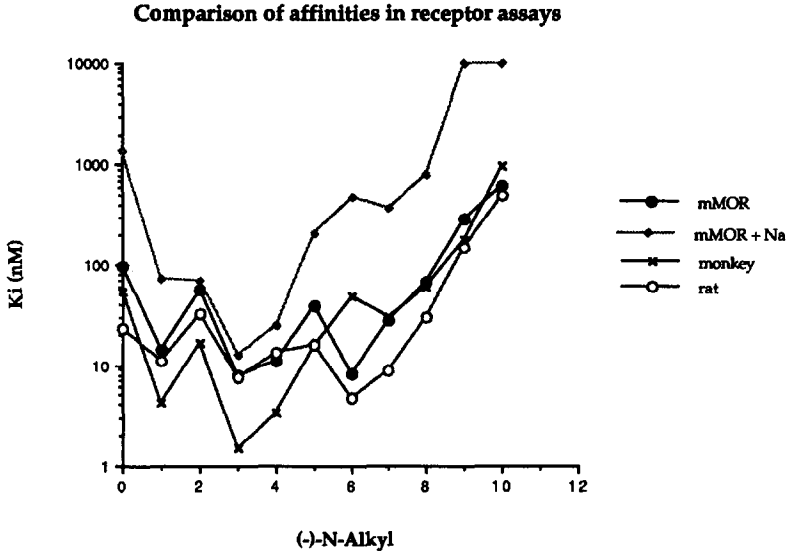


Fig. 4. Comparison of affinities (K_i, nM) for the *N*-alkyl-benzomorphans at the μ-opioid receptors. Shown are data from Table 1 as follows: mMOR cells in the absence (●) and presence (◇) of sodium as calculated by the displacement of [^{3}H] diprenorphine; and from rhesus monkey cortex membranes (x) and rat brain minus cerebellum (○) by displacement of [^{3}H]DAMGO [1].

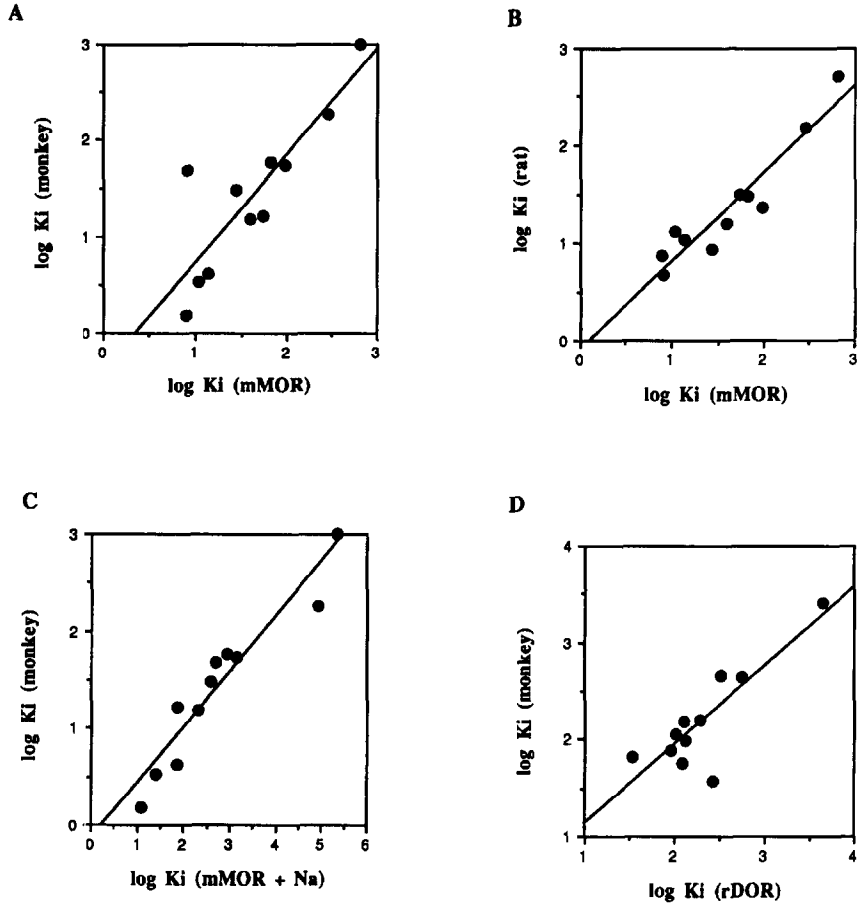


Fig. 5. Linear correlations of the affinities for the *N*-alkyl-benzomorphans at cloned opioid receptors and in heterogeneous tissues (from the data in Table 1). Correlations of log K_i values (nM) for mMOR cells in the absence of sodium vs those in rhesus monkey cortex (A) and rat brain minus cerebellum (B). Correlations of log K_i values (nM) for mMOR cells in the presence of 150 mM NaCl vs those in rhesus monkey cortex (C). Compounds with K_i values > 10 μM (in the presence of sodium) were assigned values of 10 μM for the regression analysis. Correlation of log K_i values (nM) in the rDOR cell line in the absence of sodium with that in monkey cortex (D) [1]. Correlations were obtained from linear regression analysis of the logarithm of the affinities of the compounds in displacement assays. The best correlation was obtained between the rodent μ-receptors in the absence of sodium ($r^2 = 0.89$, slope = 0.90, $P < 0.0001$), although good correlations were found with the cloned mouse μ versus the monkey ($r^2 = 0.737$, slope = 1.11, $P < 0.001$ in the absence of sodium, $r^2 = 0.905$, slope = 0.575, $P < 0.0001$ in the presence of sodium), and the cloned rat δ versus the monkey ($r^2 = 0.705$, slope = 0.86, $P < 0.0005$).

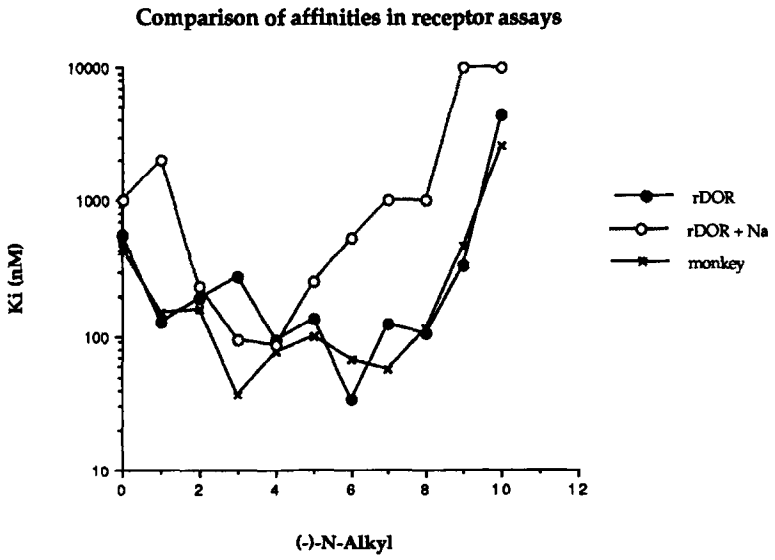


Fig. 6. Comparison of affinities (K_i, nM) for the *N*-alkyl-benzomorphans at the δ-opioid receptors. Shown are data from Table 1 as follows: rDOR cells in the absence (●) and presence (○) of sodium as calculated from the displacement of [^{3}H] diprenorphine; and in rhesus monkey cortex membranes (x) as obtained from the displacement of [^{3}H]DPDPE [1]. Compounds with K_i values > 1 μM and > 10 μM (in the presence of sodium) were assigned values of 1 μM and 10 μM for data analysis.

0.05 and 1.05 ± 0.05 for the mMOR and rDOR cell lines, respectively).

A primary focus of this investigation was to evaluate the feasibility of the CHO expression system in a series of opioid-active compounds for which the pharmacology and receptor selectivity have been defined recently [1]. The structure of the *N*-alkyl-benzomorphans used in this study is shown in Fig. 3. In addition, known δ- and μ-selective compounds were tested. Competition assays were performed with 1 nM [^{3}H]diprenorphine and six concentrations (0.1 nM to 10 μM) of displacing ligands in the absence or presence of 150 mM NaCl. The data are summarized in Table 1.

The mMOR cell line showed appropriate μ-opioid receptor selectivity, as shown by the K_i values for morphine (8.8 nM) and DAMGO (2.02 nM). Similarly, the rDOR cell line had K_i values for DPDPE (26.7 ± 2.3 nM) and deltorphin (1.8 ± 2.2 nM) consistent with that of a δ-opioid receptor (Table 1). Conversely, DAMGO did not compete well with [^{3}H]diprenorphine in the rDOR cell line, nor did DPDPE compete in the mMOR cell line (K_i values > 2 μM and > 10 μM, respectively; data not shown).

Comparison of the K_i values for the benzomorphan series in the mMOR cell line with those obtained using [^{3}H]DAMGO in monkey cortex or rat brain minus cerebellum showed similar, yet distinct profiles. The rank order of the affinities at the (mouse) μ-receptor in the mMOR cells (in the absence of sodium) was C3 = C6 > C4 > C1 > C7 > C5 > C2 > C8 > C0 > C9 > C10, as compared with that in the monkey cortex (C3 > C4 = C1 > C5 = C2 > C7 > C6 > C0 > C8 > C9 > C10) or rat brain (C6 > C3 > C7 > C1 > C4 > C5 > C0 > C8 = C2 > C9 > C10). As shown in Fig. 4, binding affinities in the mMOR cell line (in the absence of sodium) corresponded well with those in the rat brain for most of the compounds, the exception being the (−)-H substitution, for which the K_i is closer to that obtained in the monkey brain membranes. Regression analysis of the affinities of the benzomorphans to inhibit [^{3}H]diprenorphine binding in the mMOR cell line versus that obtained in displacement studies in rat brain using [^{3}H]DAMGO revealed a very good correlation (r^2 = 0.89, slope = 0.90, P < 0.0001, Fig. 5B). A good correlation was found in K_i values across species as well (r^2 = 0.737, slope = 1.11, P < 0.001, in monkey brain vs mMOR, Fig. 5A).

The K_i values obtained for the benzomorphans in the rDOR cell line (in the absence of sodium) were similar to those obtained using [^{3}H]DPDPE in monkey cortex, except for the propyl, hexyl and heptyl compounds (Table 1, Fig. 6). The rank order of the affinities at the rat δ-opioid receptor was C6 > C4 > C8 > C7 = C1 > C5 > C2 > C3 > C9 > C0 > C10 compared with that obtained in the monkey cortex (C3 > C7 = C6 = C4 > C5 > C8 > C1 = C2 > C0 = C9 > C10). Nonetheless, regression analysis of the affinities of the benzomorphans for inhibition of [^{3}H]diprenorphine binding in the rDOR cell line versus those obtained from displacement studies in monkey cortex using [^{3}H]DPDPE showed a good correlation (r^2 = 0.705, slope = 0.86, P < 0.0005, Fig. 5D).

An altered profile was observed when comparing the affinities of the benzomorphan series in the mMOR and rDOR cell lines in the presence of 150 mM NaCl. Sodium reduces the affinity of opioid agonists without affecting that of antagonists [24]. The transfected μ- and δ-opioid receptor cell lines also exhibited differential agonist and antagonist sensitivity to sodium (Table 1, Figs. 4–6). The addition of NaCl (at a final concentration of 150 mM) to the binding buffer resulted in a 2- to 10-fold increase in the K_i values for the benzomorphan compounds previously shown to be agonists, whereas the K_i values for the antagonists were altered only slightly. Sodium also reduced affinities of the compounds that were inactive in the tail flick assay (Table 1). Sodium did not alter [^{3}H]diprenorphine binding (data not shown). In the study by May *et al* [1], binding in the

monkey cortex membranes was performed in the presence of 150 mM NaCl. When comparing the affinities in the receptor assays, a better correlation with μ-binding in the monkey cortex was found with mMOR binding in the presence of sodium than in the absence of sodium (r^2 = 0.905, slope = 0.575, P < 0.0001; r^2 = 0.737, slope = 1.11, P < 0.001, respectively, Fig. 5). However, the overall affinities were more similar in the absence of sodium (Table 1, Figs. 4 and 6). Furthermore, the addition of sodium did not improve the correlation of binding affinities in the rDOR cell line as compared with that in the monkey cortex (r^2 = 0.600, slope = 0.582, P < 0.01, data not shown).

A different profile was generated when comparing the affinities of the benzomorphans at the μ- and δ-opioid receptors with their *in vivo* potencies in the mouse tail flick latency assay. Regression analysis of the K_i values of the benzomorphans to inhibit [^{3}H]diprenorphine binding in the mMOR cell line (in the absence of sodium) versus the ED_{50} values as agonists or antagonists in the tail flick latency assay revealed an excellent correlation (r^2 = 0.829, slope = 1.0, P < 0.0001, Fig. 7A) The correlations obtained from comparing the K_i values obtained from the displacement of [^{3}H]DAMGO in rat brain or monkey cortex with ED_{50} values in the mouse tail flick assay were lower (r^2 = 0.661, slope = 0.94, p < 0.005, and r^2 = 0.695, slope = 0.71, P < 0.005; Fig. 7 B and C, respectively), as were those obtained with the mMOR cell line in the presence of 150 mM NaCl (r^2 = 0.696, slope = 0.43, P < 0.005, data not shown). Correlation of K_i values obtained from displacement of [^{3}H]-DPDPE in monkey brain cortex with potency in the mouse tail flick assay were also reasonable (r^2 = 0.699, slope = 1.11, P < 0.005, data not shown). On the other hand, a much poorer correlation was found when comparing the affinities of the benzomorphans at the cloned δ-opioid receptor with *in vivo* potencies (r^2 = 0.405, slope = 0.812, P < 0.05, in the absence of sodium; r^2 = 0.492, slope = 0.69, P < 0.05, in the presence of sodium, data not shown). The data were also examined using non-parametric statistics (rank order correlations), and similar results were obtained. These data suggest that binding affinity at the cloned mouse μ-opioid receptor is the best predictor of *in vivo* antinociceptive activity in the mouse tail flick assay, at least in this class of compounds. Furthermore, the observed agonist and antagonist activities may be most likely attributed to μ-opioid receptor activation.

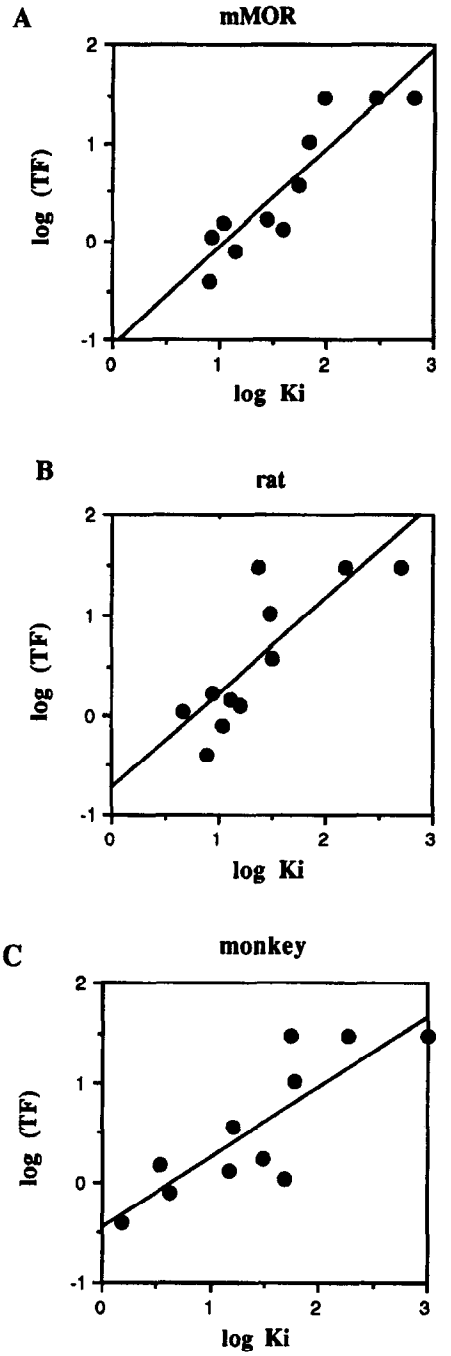


Fig. 7. Linear correlations of log ED_{50} values (mg/kg) for benzomorphans in the mouse tail flick assay expressed as log (TF) [1] and log K_i values (nM) shown in Table 1. The potencies of the benzomorphans as agonists or antagonists were weighed equally, and inactive compounds were assigned the cut-off dose of 30 mg/kg for the analysis. The best correlation was obtained with the cloned mouse μ-receptor (r^2 = 0.83, slope = 1.0, P < 0.0001, A), although good correlations were found with the rat μ-receptor (r^2 = 0.661, slope = 0.94, p < 0.005, B), and monkey μ-receptor (r^2 = 0.695, slope = 0.71, P < 0.005, C).

DISCUSSION

The results presented in this manuscript indicate that CHO cell lines expressing cloned mouse μ- or rat δ-opioid receptors are good models for determining binding affinities for the *N*-alkyl-benzomorphan class of compounds. Binding affinities in the mMOR cell line obtained from displacement of [^{3}H]diprenorphine were closely correlated with affinities obtained from displacement of [^{3}H]DAMGO in brain membrane preparations, with one exception. Similarly, affinities in the rDOR cell line using [^{3}H]diprenorphine as the radioligand correlated well overall with those in monkey cortex using [^{3}H]DPDPE; however, three of the eleven compounds were dissimilar. It thus appears that individual cloned opioid receptors and a non-selective radioligand can be used instead of selective radioligands in heterogeneous tissues when determining affinities at specific opioid receptors. Nevertheless, there are many cases where ligands bind to more than one receptor subtype, so it will be necessary to establish which additional subtypes may

be involved by conducting binding studies in heterogeneous tissues.

Several groups have reported recently the binding characteristics of cloned opioid receptors in transfected cell lines [2–19]. We found specific binding to be linear at low protein concentrations for the transfected mouse μ- and rat δ-opioid receptor expressed in CHO cells, and used 5–15 μg of membrane protein in our assays, as others have reported [2, 3, 6]. Furthermore, CHO cells stably transfected with μ- or δ-opioid receptors displayed affinities for *N*-alkyl-benzomorphans similar to those obtained using selective opioid ligands in brain homogenates. As others have found, an excellent correlation was observed between binding in cells transfected with the μ-opioid receptor versus that in brain homogenates using selective ligands. Contrary to a previous report [6], binding affinities in the cell line transfected with the δ-opioid receptor were also closely correlated with those obtained from displacement studies in monkey cortex using [^{3}H]DPDPE. One possible explanation for this result may be that binding requirements (i.e. sites on the receptor) are different for non-selective ligands, so that they do not discriminate between δ1 and δ2 subtypes [25]. Another possibility might be that δ subtypes cannot be easily discriminated in binding assays [26] without the use of selective δ ligands and analysis of affinity states [22].

The previous studies done in monkey cortex membranes were performed in the presence of 150 mM NaCl. Under this condition, agonists have lower affinity to opioid receptors, whereas antagonists are not affected [24, 27, 28]. Since the compounds evaluated in this study include both agonists and antagonists, this may have accounted for some of the differences observed. Therefore, we repeated the displacement assays in the transfected cell lines in the presence of 150 mM NaCl. Sodium reduced the affinity of those compounds previously shown to be agonists in the mouse tail flick assay, without significantly altering the affinity of antagonists. The cloned μ- and δ-opioid receptors have formerly been shown to exhibit sodium sensitivity, presumably mediated by an aspartate residue in the second transmembrane domain [25, 29]. The correlations were improved between relative μ-binding affinities in the monkey cortex and those in the mMOR cell line in the presence of sodium. However, conducting the binding in the rDOR cell line in the presence of sodium did not improve the correlation with δ-binding in the monkey cortex. Moreover, the overall binding affinities at both the transfected μ- and δ-opioid receptors in the absence of sodium were more similar to those in heterogeneous tissue.

Comparative analysis of K_i values with *in vivo* potency in the mouse tail flick test indicated a high degree of correlation between antinociception and affinity in the μ-opioid receptor cell line. In fact, a better correlation was found with the cloned mouse μ-opioid receptor expressing cell line than with affinities at the μ-opioid receptor in rat brain or monkey cortex. The differences may reflect species selectivities, as has been postulated for the guinea pig κ receptor [18]. Elucidation of the primate μ-opioid receptor sequence in comparison with the rat, mouse and human clones will help clarify this possibility. Alternatively, since the binding data from brain are from a tissue with heterogeneous opioid receptors, the differences seen here may be a reflection of binding in a pure population of μ-opioid receptors.

Lesser correlations were found between antinociception in the mouse and affinity at the δ receptor, either in the clonal cell line or in the monkey cortex. A possible reason for the poorer correlations may be the predominance of μ-opioid receptors in the mediation of antinociception [30]. Our data suggest that binding affinity at the cloned mouse μ-opioid receptor is the best predictor of *in vivo* antinociceptive activity in the mouse tail flick assay, at least in the *N*-alkyl-benzomorphans. Finally, our data indicate that transfected CHO cell lines can provide a useful model for testing novel opioid compounds.

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