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Distinct high-affinity binding sites for benzomorphan drugs and enkephalin in a neuroblastoma–brain hybrid cell line

(multiple opiate receptors/stereospecific binding/ethylketocyclazocine/*N*-allylnorcyclazocine/neurotumor cell lines)

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ABSTRACT **The high-affinity binding of benzomorphan drugs (ethylketocyclazocine and *N*-allylnorcyclazocine) and [DAla^2,DLeu^5]enkephalin was examined in a mouse neuroblastoma–Chinese hamster brain clonal hybrid cell line (NCB-20). Scatchard analysis of saturation binding isotherms indicated the presence of a single binding site for ^{3}H-labeled [DAla^2,DLeu^5]enkephalin (K_d = 3 nM) and multiple binding sites for [^{3}H]ethylketocyclazocine (K_d = 4 and 20 nM) and *N*-[^{3}H]allylnorcyclazocine (K_d = 0.5 and 15 nM). Both ethylketocyclazocine and *N*-allylnorcyclazocine competed (K_i = 10 and 30 nM, respectively) with [^{3}H][DAla^2,DLeu^5]enkephalin binding in NCB-20 cells but neither [DAla^2,DLeu^5]enkephalin nor morphine could completely inhibit the specific binding of [^{3}H]ethylketocyclazocine (7 nM) or *N*-[^{3}H]allylnorcyclazocine (3 nM). Furthermore, not all benzomorphan drugs (e.g., ethylketocyclazocine) were totally efficacious in displacing 3 nM *N*-[^{3}H]allylnorcyclazocine binding in the presence or absence of high concentrations of [DAla^2,DLeu^5]enkephalin. The data presented suggest that benzomorphan drugs interact with three distinct high-affinity binding sites: (*i*) a site that binds enkephalin and morphine in addition to ethylketocyclazocine and *N*-allylnorcyclazocine; (*ii*) a site that binds *both* ethylketocyclazocine and *N*-allylnorcyclazocine but *not* enkephalin and morphine; and (*iii*) a site that binds *N*-allylnorcyclazocine but *not* enkephalin, morphine, or ethylketocyclazocine. The first of these sites was comparable to the δ opiate receptor expressed in NG108-15 and N4TG1 cell lines based on the potency series obtained for various opiates and benzomorphan drugs in competition studies with [^{3}H][DAla^2,DLeu^5]-enkephalin. However, the specific high-affinity benzomorphan binding sites thus far are unique and may represent biochemical correlates of κ and σ opiate receptors which have been proposed to exist on the basis of physiological studies.**

Physiological studies (1–3) have suggested that opiates and related narcotic drugs (benzomorphans) may have four distinct sites of action in the central nervous system—the so-called δ, μ, κ, and σ opiate receptors. However, attempts to resolve this growing complexity biochemically by differential binding studies have been hindered by the heterogeneous nature of nerve tissue. Some success has been reported in differentiating separate classes of opiate receptors on the basis of effects of sodium, GTP, and divalent cations (4–8) but direct evidence of discrete receptor subpopulations has primarily come from studies in peripheral tissue preparations and clonal neurotumor cell lines (9, 10) enriched in a single high-affinity opiate binding site.

It is now generally accepted that opiate receptors (μ subtype) that respond preferentially to morphine may be found in guinea pig ileum (1), whereas opiate receptors (δ subtype) that respond preferentially to enkephalin are in high concentration in the mouse vas deferens (1) and in mouse neuroblastoma N4TG1 and mouse neuroblastoma–rat glioma NG108-15 cells (4, 9, 10). In addition, the coexistence of δ and μ receptors in rat brain homogenates has been recently demonstrated (11) by the use of phenoxybenzamine to inactivate unprotected binding sites for either enkephalin or morphine irreversibly. Thus, the existence of physically distinct enkephalin (δ) and morphine (μ) receptors appears to be established.

In contrast, the concept of specific receptors that respond preferentially to benzomorphan drugs (κ and σ subtypes), as originally proposed by Martin and colleagues (2, 3) to explain the different physiological effects of ethylketocyclazocine, ketocyclazocine, and *N*-allylnorcyclazocine, has not gained widespread acceptance. In fact, there has been little biochemical evidence to date in support of such a concept and it has been suggested that δ and μ opiate receptors may alone mediate the actions of benzomorphan drugs (6–8, 12).

Such a two-site model (δ and μ) does not adequately account for the diverse pharmacological properties of opiates and benzomorphans. In this paper we present biochemical evidence for the interaction of benzomorphan drugs with high-affinity binding sites that are distinct from receptors for enkephalin and morphine.

MATERIALS AND METHODS

[^{3}H]Ethylketocyclazocine (15 Ci/mmol; 1 Ci = 3.7 × 10^{10} becquerels), *N*-[^{3}H]allylnorcyclazocine (33 Ci/mmol), and [^{3}H][DAla^2,DLeu^5]enkephalin (27 Ci/mmol) were purchased from New England Nuclear. The following drugs were generous gifts: morphine sulfate, etorphine, and naloxone hydrochloride, from B. Wainer (University of Chicago); *N*-allylnorcyclazocine, ethylketocyclazocine, and the benzomorphan stereoisomers UM1071-R and UM 1071-S, J. H. Woods (University of Michigan); cyclazocine and ketocyclazocine, R. S. Zukin (Albert Einstein College of Medicine); and [DAla^2,DLeu^5]enkephalin, S. Wilkinson (Burroughs Wellcome). Bremazocine was obtained from Sandoz and WIN 44,441-3 was from Sterling Winthrop.

Neurotumor hybrid cell lines NCB-20 and NG108-15 were generous gifts from J. Minna (Veterans Administration Hospital, Washington, DC) and W. A. Klee (National Institutes of Health, Bethesda, MD), respectively. Cells were either grown as monolayer cultures on 100-mm Falcon plastic dishes as described (13) or adapted to growth on 670-cm^2 borosilicate tissue culture roller bottles (Bellco Glass) in modified Eagle's medium supplemented with 10% fetal calf serum and an atmosphere of 90% air/10% CO_2. Harvested cells were allowed to swell in 50 mM Tris·HCl (pH 7.4) for 30 min prior to disruption with a Polytron PT-20 homogenizer, and crude membrane fractions were recovered by centrifugation at 50,000 × *g* for 30 min.

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Binding assays were performed via a modification of the procedure described by Chang *et al.* (10). Aliquots of crude membrane preparations [0.2 ml; 0.1–0.5 mg of protein as determined by the Lowry (14) procedure] in 50 mM Tris·HCl (pH 7.4) were incubated for 45 min at 25°C with various concentrations of radiolabeled ligand and unlabeled drug (final incubation volume 0.24 ml.) One milliliter of ice-cold 50 mM Tris·HCl (pH 7.4) was added to each sample prior to filtration through Whatman GF/B glass microfiber filters under vacuum, the filters were washed twice with 5.0 ml of ice-cold buffer, and the bound radioactivity was determined by liquid scintillation counting. Nonspecific binding was measured in the presence of a large excess (1–100 μM) of the respective unlabeled ligand. Data used for calculating K_d, K_i, concentration for 50% inhibition of effect (IC_{50}), and B_{max} values were means of triplicate determinations with variation between individual determinations (based on 40% counting efficiency) never exceeding 5%.

RESULTS

Scatchard Analysis of [^{3}H][DAla^2,DLeu^5]Enkephalin Binding. Saturable, high-affinity binding of [^{3}H][DAla^2,DLeu^5]enkephalin was observed in membrane preparations of neurotumor hybrid cell lines NCB-20 and NG108-15. Scatchard plots of saturation binding isotherms were linear (Fig. 1) and suggested the presence of a single high-affinity binding site for [^{3}H][DAla^2,DLeu^5]enkephalin in each cell line. The affinities of these binding sites were similar in the two cell lines (K_d = 3 nM), but the total number of sites in membrane preparations of NCB-20 cells (B_{max} = 400 fmol/mg of protein) was 4-fold higher than that found in NG108-15 cells (B_{max} = 90 fmol/mg of protein).

Scatchard Analysis of [^{3}H]Ethylketocyclazocine Binding. A nonlinear Scatchard plot was obtained for the specific binding of [^{3}H]ethylketocyclazocine in NCB-20 cell homogenates (Fig. 2*A*) and could be resolved into two linear components. There were 3–4 times as many apparent low-affinity binding sites (K_d = 20 nM; B_{max} = 1000 fmol/mg of protein) as there were apparent high-affinity binding sites (K_d = 4 nM; B_{max} = 300 fmol/mg of protein). In contrast, only a single high-affinity binding site for [^{3}H]ethylketocyclazocine (K_d = 5 nM; B_{max} = 70 fmol/mg of protein) could be identified on the basis of Scatchard analysis in NG108-15 hybrid cells (Fig. 2*B*).

Scatchard Analysis of *N*-[^{3}H]Allylnorcyclazocine Binding. The saturable binding of *N*-[^{3}H]allylnorcyclazocine in NCB-20 cells was also characterized by a nonlinear Scatchard plot (Fig. 3*A*) and was consistent with the expression of multiple binding sites for benzomorphan drugs. Two linear components could be resolved, an apparent high-affinity binding site (K_d = 0.5 nM; B_{max} = 100 fmol/mg of protein) and an apparent low-affinity binding site (K_d = 15 nM; B_{max} = 1000 fmol/mg of protein). In NG108-15 cell homogenates (Fig. 3*B*) *N*-[^{3}H]allylnorcyclazocine was found to interact with only a single bind-

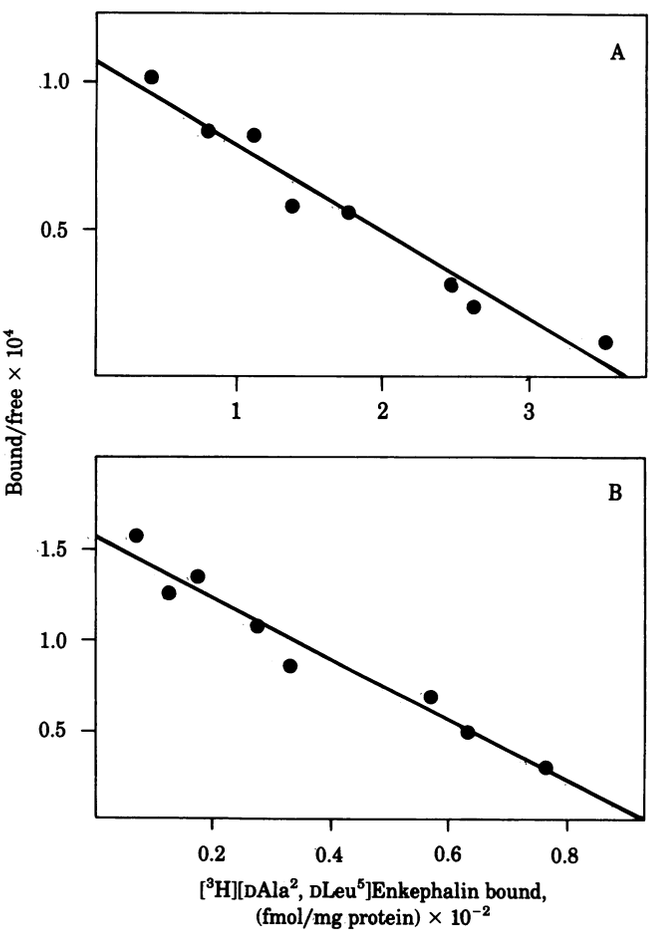

FIG. 1. Scatchard analysis of [^{3}H][DAla^2,DLeu^5]enkephalin binding in NCB-20 (*A*) and NG108-15 (*B*) hybrid cells. Membrane preparations were incubated with [^{3}H][DAla^2,DLeu^5]enkephalin in the concentration range 0.3–20 nM and specific binding was assayed. Nonspecific binding was measured in the presence of 100 μM [DAla^2,DLeu^5]enkephalin.

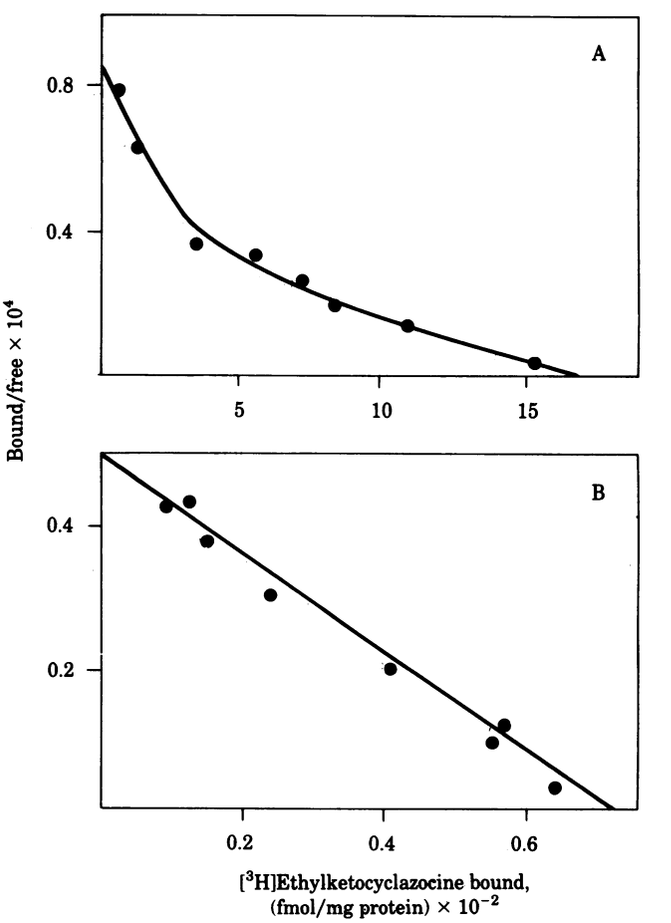

FIG. 2. Scatchard analysis of [^{3}H]ethylketocyclazocine binding in NCB-20 (*A*) and NG108-15 (*B*) hybrid cells. Membrane preparations were incubated with [^{3}H]ethylketocyclazocine in the concentration range 0.5–100 nM and specific binding was assayed. Nonspecific binding was measured in the presence of 100 μM ethylketocyclazocine.

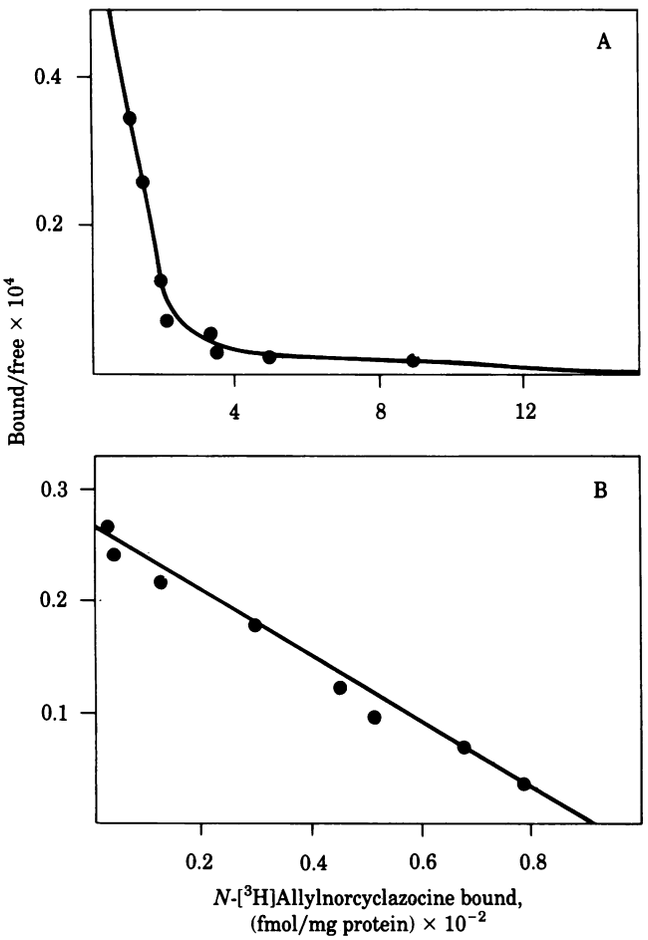

FIG. 3. Scatchard analysis of *N*-[^{3}H]allylnorcyclazocine binding in NCB-20 (*A*) and NG108-15 (*B*) hybrid cells. Membrane preparations were incubated with *N*-[^{3}H]allylnorcyclazocine in the concentration range 0.2–100 nM and specific binding was assayed. Nonspecific binding was measured in the presence of 100 μM *N*-allylnorcyclazocine.

ing site having an affinity (K_d = 20 nM; B_{max} = 90 fmol/mg of protein) comparable to that of the apparent low-affinity site in NCB-20 cells.

Inhibition of [^{3}H][DAla^2,DLeu^5]Enkephalin Binding. A wide range of opiate and benzomorphan drugs competed for high-affinity [^{3}H][DAla^2,DLeu^5]enkephalin binding sites in NG108-15 and NCB-20 hybrid cells (Table 1). The relative potencies of these drugs in displacing 4 nM [^{3}H][DAla^2,DLeu^5]enkephalin were essentially the same in the two cell lines ([DAla^2,DLeu^5]enkephalin = etorphine = bremazocine ≥ WIN 44,441-3 > ethylketocyclazocine > cyclazocine ≥ *N*-allylnorcyclazocine > ketocyclazocine > morphine = naloxone), implying that the [^{3}H][DAla^2,DLeu^5]enkephalin binding site in NCB-20 was identical to the previously characterized (4) δ opiate receptor in NG108-15. In general, benzomorphans were better competitors than morphine (a μ agonist) but somewhat less potent than [DAla^2,DLeu^5]enkephalin (a δ agonist)—however, both the putative κ agonist bremazocine and the putative κ antagonist WIN 44,441-3 had high affinities for [^{3}H][DAla^2,DLeu^5]enkephalin binding sites. The apparent K_i obtained for ethylketocyclazocine and *N*-allynorcyclazocine (Table 1) were in close agreement with K_d determined by Scatchard analysis of saturation binding isotherms (Figs. 2 and 3), indicating that both [^{3}H]ethylketocyclazocine and *N*-[^{3}H]allylnorcyclazocine were binding to the same high-affinity site (δ receptor) in NG108-15 cells as [^{3}H][DAla^2,DLeu^5]enkephalin.

Table 1. Potencies of enkephalin, opiates, and benzomorphans for inhibiting [^{3}H][DAla^2,DLeu^5]enkephalin binding in NG108-15 and NCB-20 hybrid cells

Competitor	K_i, nM NG108-15	K_i, nM NCB-20
[DAla^2,DLeu^5]Enkephalin	1	1
Etorphine	4	0.2
Morphine	90	100
Naloxone	200	100
Cyclazocine	9	20
N-Allylnorcyclazocine	40	30
Ketocyclazocine	40	50
Ethylketocyclazocine	10	10
Bremazocine	2	1
WIN 44,441-3	0.5	5

Apparent dissociation constants (K_i) were calculated according to the equation of Cheng and Prusoff (15), $K_i = IC_{50}/[1 + ([L]/K_d)]$ in which [L] is the concentration of radiolabeled ligand, K_d is the dissociation constant (3 nM) for the radiolabeled ligand, and IC_{50} values are the concentrations of competing drugs required to inhibit the displaceable binding of radiolabeled ligand by 50%. The binding of 4 nM [^{3}H][DAla^2,DLeu^5]enkephalin in membrane preparations was assayed in the presence or absence of increasing concentrations of competing drugs. Nonspecific binding was measured in the presence of 100 μM [DAla^2,DLeu^5]enkephalin.

Inhibition of [^{3}H]Ethylketocyclazocine and *N*-[^{3}H]Allylnorcyclazocine Binding. The competitive effects of enkephalin, opiates, and benzomorphans against 7 nM [^{3}H]ethylketocyclazocine and 3 nM *N*-[^{3}H]allylnorcyclazocine were studied in NCB-20 hybrid cells. At these concentrations, radiolabeled benzomorphan derivatives were directed equally toward apparent high- and low-affinity binding sites and it was therefore possible to investigate drug interactions at multiple sites simultaneously. The drug potency series against [^{3}H]ethylketocyclazocine and *N*-[^{3}H]allylnorcylazocine binding (Table 2) was markedly different from that seen with 4 nM [^{3}H][DAla^2,DLeu^5]

Table 2. Potencies of enkephalin, opiates, and benzomorphans for inhibiting [^{3}H]ethylketocyclazocine and *N*-[^{3}H]allylnorcyclazocine binding in NCB-20 hybrid cells

Competitor	IC_{50}, nM [^{3}H]Ethylketocyclazocine	IC_{50}, nM *N*-[^{3}H]Allylnorcyclazocine
[DAla^2,DLeu^5]Enkephalin	>10^5 (0.3)	>10^5 (3)
Etorphine	>10^5 (0.3)	>10^5 (0.4)
Morphine	>10^5 (40)	>10^5 (80)
Naloxone	10^5	10^5
Cyclazocine	100	100
N-Allylnorcyclazocine	200	20
Ketocyclazocine	40	30
Ethylketocyclazocine	20	25*
Bremazocine	25	200
WIN 44,441-3	25 × 10^3	10^5

The binding of 7 nM [^{3}H]ethylketocyclazocine and 3 nM *N*-[^{3}H]allylnorcyclazocine in membrane preparations was assayed in the presence or absence of increasing concentrations of competing drugs. Nonspecific binding was measured in the presence of 100 μM ethylketocyclazocine and 100 μM *N*-[^{3}H]allylnorcyclazocine. Values in parenthesis represent IC_{15} values because radiolabeled ligand maximally displaced by competing drug was less than 30% of the radiolabeled ligand specifically bound.

* This is an IC_{38} value because radiolabeled ligand maximally displaced by competing drug was less than 75% of the radiolabeled ligand specifically bound.

enkephalin (Table 1), suggesting the presence of binding sites distinct from δ receptors. Of particular interest was the observation that at least 4000-fold higher concentrations of WIN 44,441-3 were required to inhibit the specific binding of [^{3}H]benzomorphans than the binding of [^{3}H][DAla^2,DLeu^5]enkephalin. Furthermore, putative κ agonists (ethylketocyclazocine, ketocyclazocine, and bremazocine) were more potent than putative σ agonists (*N*-allylnorcyclazocine and cyclazocine) in displacing [^{3}H]ethylketocyclazocine binding in these cells. In contrast, bremazocine and cyclazocine were only weak competitors of 3 nM *N*-[^{3}H]allylnorcyclazocine whereas *N*-allylnorcyclazocine, ethylketocyclazocine, and ketocyclazocine were strong competitors. Only high concentrations of the opiate antagonist naloxone could block the binding of either [^{3}H]ethylketocyclazocine and *N*-[^{3}H]allylnorcyclazocine.

Differences in the efficacies of certain drugs (Table 2) in displacing [^{3}H]ethylketocyclazocine and *N*-[^{3}H]allylnorcyclazocine binding provided additional evidence for the interaction of benzomorphan drugs with binding sites distinct from δ receptors in NCB-20 cells. The opiate agonists [DAla^2,DLeu^5]enkephalin, etorphine, and morphine failed to displace more than 30% of either [^{3}H]benzomorphan derivative in competition studies (Table 2). Good correlation of IC_{15} values obtained for these drugs with apparent K_i in studies with [^{3}H][DAla^2, DLeu^5]enkephalin (Table 1) suggested that the displaceable components of [^{3}H]ethylketocyclazocine and *N*-[^{3}H]allylnorcyclazocine binding corresponded to interactions with enkephalin (δ) receptors, whereas the nondisplaceable components represented binding to other sites. Although *N*-allylnorcyclazocine completely inhibited the specific binding of [^{3}H]ethylketocyclazocine in NCB-20 cells (Table 2), ethylketocyclazocine could only displace 75% of 3 nM *N*-[^{3}H]allylnorcyclazocine either in the presence or absence of 100 μM [DAla^2,DLeu^5]enkephalin. This finding further implied that one of the binding sites not shared by enkephalin, etorphine, or morphine also had little affinity for ethylketocyclazocine.

The binding of benzomorphan derivatives in NCB-20 cells was stereospecific (Fig. 4) in that a 1000-fold difference in potency was observed for the stereoisomers UM 1071-R (IC_{50} = 10 nM) and UM 1071-S (IC_{50} = 10,000 nM) in competition binding studies with 3 nM *N*-[^{3}H]allylnorcyclazocine. This agrees with previous reports (16) that only the *R* enantiomer is active *in vivo*.

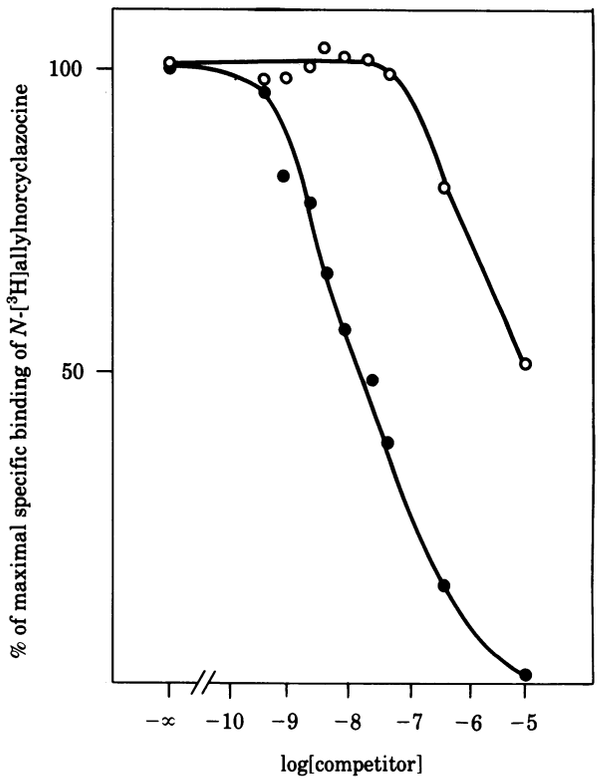

FIG. 4. Inhibition of the specific binding of *N*-[^{3}H]allylnorcyclazocine in NCB-20 hybrid cells by the benzomorphan stereoisomers UM 1071-R (●) and UM 1071-S (○). The binding of 3 nM *N*-[^{3}H]allylnorcyclazocine in membrane preparations was assayed in the presence or absence of increasing concentrations of competing drug. Nonspecific binding was measured in the presence of 100 μM *N*-allylnorcyclazocine.

DISCUSSION

Benzomorphans are an enigmatic group of opioid drugs which produce a morphine-like depression of the nociceptive response yet fail to suppress withdrawal symptoms in morphine-dependent animals (2, 3, 16–19). Detailed studies of the differential response to opiates and benzomorphans in dogs led Martin and coworkers (2, 3) to propose the existence of three discrete subpopulations of opiate receptors—μ, κ, and σ. The prototypical μ agonist morphine both suppressed abstinence and produced analgesia in morphine-dependent dogs, whereas ethylketocyclazocine and ketocyclazocine neither suppressed nor precipitated abstinence in morphine-dependent animals but did produce naloxone-reversible analgesia (2, 3). It was therefore concluded that ethylketocyclazocine has a site of action, designated as the κ receptor, distinct from morphine (μ) receptors. Another type of naloxone-reversible effect—behavioral excitation without inducing analgesia or depressing abstinence in morphine- and ketocyclazocine-dependent dogs—was observed (2) with the benzomorphan derivative *N*-allylnorcyclazocine (SKF 10,047). To explain this, the site mediating this response was designated the σ receptor. Behavioral studies in monkeys, rats, and mice (16–19) and electrophysiological studies in guinea pig ileum preparations (1, 19) have provided support for the concept that benzomorphans, such as ethylketocyclazocine and bremazocine, may exert their pharmacological actions at sites other than those previously characterized as morphine (μ) and enkephalin (δ) receptors.

In this investigation we have demonstrated that benzomorphan drugs interact stereospecifically with multiple binding sites in a somatic cell hybrid NCB-20. One of the high-affinity binding sites appears to be identical to the enkephalin (δ) receptor found in neurotumor cell lines NG108-15 and N4TG1 (4, 9, 10), rat brain homogenates (1, 4, 11), and mouse vas deferens (1). The other high-affinity sites expressed in NCB-20 cells, which bind benzomorphan drugs preferentially, have not been previously described. Both ethylketocyclazocine (a putative κ agonist) and *N*-allylnorcyclazocine (a putative σ agonist) bind to a site which is not occupied by enkephalin or the classical opiate agonists morphine and etorphine. Furthermore, *N*-allylnorcyclazocine binds to a separate site having little affinity for enkephalin, morphine, etorphine, or ethylketocyclazocine. Based on differences in the selectivity of the two specific benzomorphan binding sites for ethylketocyclazocine, it may not be unreasonable to suggest that these sites are equivalent to the κ and σ opiate receptor subtypes defined by Martin *et al.* (2).

Additional support for the contention that specific receptors for benzomorphan drugs do exist comes from the observation that [^{3}H]ethylketocyclazocine binds to what has been tentatively called a κ site in guinea pig brain homogenates (20). The binding of [^{3}H]ethylketocyclazocine was saturable, biphasic, and readily displaceable by etorphine. However, low concentrations of morphine and [DAla^2,DLeu^5]enkephalin only displaced 35% of [^{3}H]ethylketocyclazocine bound, implying that

this ligand was interacting mainly with κ sites (i.e., the 65% of total binding not displaced) and to a lesser extent with μ and δ sites. Except that we do not find any evidence that etorphine can displace [^{3}H]ethylketocyclazocine or *N*-[^{3}H]allylnorcyclazocine from specific benzomorphan binding sites in NCB-20 cells, our data are consistent with these findings (20).

Our observations clearly indicate the presence of discrete benzomorphan receptor subpopulations in nerve tissue. However, a number of recent reports (6–8, 12) have failed to provide any corroborative evidence for the existence of κ and σ opiate receptors. Chang *et al.* (6) studied binding of ^{125}I-labeled [DAla^2,DLeu^5]enkephalin and [^{3}H]ethylketocyclazocine in rat brain homogenates and found no evidence for the interaction of benzomorphan drugs with sites other than those previously characterized as enkephalin (δ) and morphine (μ) receptors. Therefore, they suggested that the analgesic effects of putative κ agonists (e.g., ethylketocyclazocine) may be mediated through μ receptors and that the behavioral effects of putative σ agonists (e.g., *N*-allylnorcyclazocine) may be mediated through δ receptors. In independent studies, Pasternak (7) and Hiller and Simon (12) both concluded that high-affinity [^{3}H]ethylketocyclazocine binding sites in ratbrain homogenates resembled morphine receptors. Pasternak found that sodium ions, divalent cations, and GTP affect [^{3}H]ethylketocyclazocine binding in a manner analogous to that seen with [^{3}H]morphine; Hiller and Simon noted similarities in the regional distribution of [^{3}H]-ethylketocyclazocine and [^{3}H]naloxone binding. Snyder and Goodman (8) also failed to identify unique receptors for benzomorphan drugs; they proposed that high-affinity binding of [^{3}H]ethylketocyclazocine and *N*-[^{3}H]allylnorcyclazocine corresponded to interactions with μ sites and that low-affinity binding occurred at δ sites.

We would agree that benzomorphan drugs may exert some of their effects through enkephalin (δ) and morphine (μ) receptors, but a two-site model (δ and μ) cannot explain all of the pharmacological properties associated with this unique group of narcotics. Specifically, a two-site model cannot explain why benzomorphans fail to suppress or precipitate abstinence in morphine-dependent animals (2, 3, 16–19). Benzomorphan drugs must have sites of action that are distinct from those of enkephalin and morphine. We have presented evidence for their existence in a neurotumor hybrid cell line.

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1. Lord, J. A. H., Waterfield, A. A., Hughes, J. & Kosterlitz, H. W. (1977) *Nature (London)* **267**, 495–499.
2. Martin, W. R., Eades, C. G., Thompson, J. A., Huppler, R. E. & Gilbert, P. E. (1976) *J. Pharmacol. Exp. Ther.* **197**, 517–532.
3. Gilbert, P. E. & Martin, W. R. (1976) *J. Pharmacol. Exp. Ther.* **198**, 66–82.
4. Chang, K.-J. & Cuatrecasas, P. (1979) *J. Biol. Chem.* **254**, 2610–2618.
5. Pert, C. B. & Taylor, D. (1979) in *Endogenous and Exogenous Opiate Agonists and Antagonists*, ed. Way, E. L. (Pergamon, New York), pp. 87–90.
6. Chang, K.-J., Hazum, E. & Cuatrecasas (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4469–4473.
7. Pasternak, G. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3691–3694.
8. Snyder, S. H. & Goodman, R. R. (1980) *J. Neurochem.* **35**, 5–15.
9. Klee, W. A. & Nirenberg, M. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 3474–3477.
10. Chang, K.-J., Miller, R. J. & Cuatrecasas, P. (1978) *Mol. Pharmacol.* **14**, 961–970.
11. Robson, L. E. & Kosterlitz, H. W. (1979) *Proc. R. Soc. London* **205**, 425–432.
12. Hiller, J. M. & Simon, E. J. (1980) *J. Pharmacol. Exp. Ther.* **214**, 516–519.
13. Dawson, G., Matalon, R. & Dorfman, A. (1972) *J. Biol. Chem.* **247**, 5944–5950.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
15. Cheng, Y. C. & Prusoff, W. H. (1973) *Biochem. Pharmacol.* **22**, 3099–3108.
16. Hein, D. W., Young, A. M., Herling, S. & Woods, J. H. (1981) *J. Pharmacol. Exp. Ther.*, in press.
17. Swain, H. H. & Seevers, M. H. (1976) *Addendum to the Proceedings of the 38th Annual Scientific Meeting of the Committee on Problems of Drug Dependence*, Vol. 2, pp. 768–787.
18. Teal, J. J. & Holtzman, S. G. (1980) *Eur. J. Pharmacol.* **68**, 1–10.
19. Romer, D., Buscher, D., Hill, R. C., Maurer, R., Petcher, T. J., Welle, H. B. A., Bakel, H. C. C. K. & Akkerman, A. M. (1980) *Life Sci.* **27**, 971–978.
20. Kosterlitz, H. W. & Paterson, S. J. (1980) *Proc. R. Soc. London* **210**, 113–122.