rabbit immunoglobulin), whereas L cells treated with normal rabbit serum showed no fluorescence (*6*). Further immunofluorescence studies showed the presence of cell-bound rabbit immunoglobulin with all of the cell-antiserum combinations with which stimulation occurs.

Other studies (*6*) indicate that the large increases in radioactive nucleoside uptake may be due in part to increased nucleoside transport, but it is clear from the measurements of cell number that cell proliferation is also being stimulated. There is a close analogy here to the stimulatory effects of lectins on lymphocyte cell growth, in which there are marked increases in radioactive [^{3}H]dT uptake with smaller increases in cell number and total cellular DNA content.

Our tentative conclusions are that antibodies specific for cell surface antigens can induce the cell to undergo DNA synthesis and cell division and that the antigenic determinants involved may be similar on the several different cell lines that show immunologic cross-reactivity. One of these cell lines, L-929, was transformed by a chemical carcinogen, methylcholanthrene, yet shows cross-stimulation with antiserums against two human cancers that have no known transforming agent. In cell lines stimulated by the same antiserums, the cross-stimulation we see could be due to the presence of common, tumor-related glycopeptides, but the possibility of viral or mycoplasmal infection of our cultures should also be considered. Viral infection is highly unlikely because we have obtained comparable immune stimulation with fresh cell lines in another laboratory with different equipment. Mycoplasma-free and mycoplasma-infected cell lines yield similar immune stimulation.

This is, to our knowledge, the first direct evidence that antibodies can stimulate the growth of transformed, nonlymphocytic cell lines. While the mechanism is unclear, there has been considerable speculation about the possible role of increased transport of essential nutrients into the cell during accelerated cell growth. In this connection, it is of interest that stimulation of active transport of potassium has been demonstrated in sheep LK red blood cells after incubation with sheep antibody against the L determinant (*7*). The possible relevance of these observations to blocking effects of antiserum on cell-mediated immunity in vitro (*8*) and failures in immune resistance to tumor growth in vivo deserves careful study.

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Opiate Agonists and Antagonists Discriminated by Receptor Binding in Brain

Abstract. *Receptor binding of opiate agonists and antagonists can be differentiated in vivo and in vitro. Administration of either rapidly elevates stereospecific [^{3}H]dihydromorphine binding to mouse brain extracts by 40 to 100 percent, but antagonists are 10 to 1000 times more potent than agonists; as little as 0.02 milligram of naloxone per kilogram of body weight significantly enhances opiate receptor binding. Sodium enhances antagonist binding in vitro but decreases agonist binding, a qualitative difference that may be relevant to the divergent pharmacological properties of opiate agonists and antagonists.*

Opiate antagonists are thought to occupy opiate receptor sites, preventing the access of opiates but not themselves eliciting analgesia or euphoria. Potent, long-lasting, and "pure" antagonists may provide an effective treatment for addiction, while drugs combining opiate agonist and antagonist activities offer promise as nonaddicting analgesics (*1*).

We described opiate binding in animal nervous tissue (*2*) which we attributed to specific opiate receptor sites on the basis of the stereospecificity of binding (*3*) and the close parallel between affinity for binding sites and pharmacological potency. These results have been confirmed (*4, 5*). Of 40 monkey brain regions examined, binding is most enriched in the anterior amygdala, hypothalamus, periaqueductal gray, and caudate head (*6*). Selective destruction of specific catecholamine, serotonin, and acetylcholine neural pathways in the brain does not alter binding (*6*). Receptor binding is degraded by trypsin, chymotrypsin, and phospholipase A in concentrations of less than 0.5 μg/ml and by 0.01 percent concentrations of the detergents Triton-X 100, deoxycholate, and sodium dodecyl sulfate, results suggesting that protein and lipid are important constituents of the opiate-receptor complex (*7*). The receptor is present in all vertebrate brains examined, including mammals, birds, reptiles, amphibia, and teleost fish, but cannot be detected in invertebrates such as arthropods and platyhelminths (*8*). We now report differential receptor interactions of opiate agonists and antagonists in vivo and in vitro.

Homogenates of mouse or rat brain (150 ml per gram) were incubated in triplicate in the dark for 30 minutes at 25°C in the presence of $10^{-7}M$ levorphanol or $10^{-7}M$ dextrorphan after the addition of the appropriate isotopically labeled opiate or antagonist. Samples were filtered (*2*) and washed with two 5-ml portions of tris(hydroxymethyl)-aminomethane (tris) buffer at 4°C. After extraction, the filters were counted by liquid scintillation (*2*). Specific opiate receptor activity was calculated by subtracting binding of the ^{3}H-labeled ligand in the presence of $10^{-7}M$ levorphanol from binding in the presence of $10^{-7}M$ dextrorphan, its analgeti-

cally inactive enantiomer in all experiments except those involving NaCl, in which the antagonist levallorphan and its (+) isomer were substituted for levorphanol and dextrorphan. [^{3}H]Naloxone (23.6 c/mmole), [^{3}H]oxymorphone (0.8 c/mmole), [^{3}H]levorphanol (5.4 c/mmole), [^{3}H]levallorphan (7.6 c/mmole), and [^{3}H]nalorphine (3.4 c/mmole) were obtained from New England Nuclear and purified as described for [^{3}H]naloxone (*2*). [^{3}H]-Dihydromorphine (55 c/mmole) was also purchased from New England Nuclear.

Table 1. Relative capacities of opiate antagonists and agonists to increase stereospecific [^{3}H]dihydromorphine binding to mouse brain extracts. Drugs or 0.9 percent NaCl were injected intraperitoneally, and mice were decapitated 20 minutes later. Before assaying for opiate receptor activity, it was imperative to remove virtually all the nonradioactive morphine, since low concentrations inhibit binding (*2*). Brain homogenates from control and treated mice were centrifuged at 18,000*g* for 10 minutes, the supernatant fluid was discarded, and the pellet was resuspended in 14 ml of cold tris buffer. This washing procedure was repeated, and after a third centrifugation each homogenate was suspended in 150 ml of the buffer for individual assay. The washing procedure removes virtually all of the ^{3}H in brains of mice treated in vivo with [^{3}H]naloxone or [^{3}H]oxymorphone, 1 mg/kg (14 μc). Morphine pellets (75 mg) were implanted in male Jackson mice (30 g) under light ether anesthesia in the dorsal subcutaneous space (*10*). Control mice were implated with placebo pellets composed of the inert ingredients in the morphine pellets. In later experiments, sham-operated mice in which a small incision had been made under anesthesia were used as controls. Values for placebo-implanted, sham-operated, saline-injected, and naive mice are indistinguishable, 24.6 ± 0.4 fmole of stereospecifically bound drug per milligram of protein. All increases are significant ($P < .02$) by the Mann-Whitney U rank test. Each value is the mean for one to three experiments. Groups of seven control and seven drug-treated mice were used in each experiment; individual mouse brains were assayed in triplicate with [^{3}H]dihydromorphine (1 n*M*) in the presence of levorphanol or dextrorphan (100 n*M*). Values indistinguishable from those shown were obtained when [^{3}H]naloxone was used in the binding assay; N.S., not significant.

Drug	Dose (mg/kg)	Increase (%)
Nalorphine HCl	0.05	N.S.
	0.2	37
	2.0	42
	10.0	85
Morphine SO_4	2.0	N.S.
	10.0	35
	20.0	56
	50.0	67
Naloxone HCl	0.02	11
	0.2	60
	2.0	62
	20.0	74
Oxymorphone HCl	2.0	N.S.
	10.0	N.S.
	20.0	59
Levallorphan tartrate	0.1	27
	0.5	43
Levorphanol tartrate	0.5	N.S.
	1.0	12
	2.0	30
Morphine pellet implantation		
2 hours		43
12 hours		58
24 hours		54
36 hours		40
60 hours		54
108 hours		43
156 hours		N.S.

In vivo administration of opiates and their antagonists enhances opiate receptor binding in mouse brain homogenates (Table 1). Narcotic antagonists are 10 to 1000 times more potent than their corresponding agonists in enhancing receptor binding. Thus, nalorphine increases receptor binding 37 percent at a concentration of 0.2 mg per kilogram of body weight, while morphine has no effect at 2 mg/kg and enhances binding by 35 percent at 10 mg/kg. Naloxone appears to be even more potent compared to oxymorphone, its corresponding agonist. At 0.02 mg/kg, naloxone elicits a statistically significant 11 percent elevation in receptor binding, with a 60 percent increase at 0.2 mg/kg. By contrast, oxymorphone produces no effect at 2 mg/kg or 10 mg/kg and a 59 percent rise at 20 mg/kg. Thus, while nalorphine is 10 to 50 times more potent than morphine, naloxone appears to be 100 to 1000 times more potent than oxymorphone in enhancing receptor binding. Levallorphan increases binding 27 percent at 0.1 mg/kg while levorphanol fails to alter binding at 0.5 mg/kg. Enhanced binding is observed as early as 5 minutes after morphine or naloxone treatment and disappears after 2 hours. Measurements of receptor binding at different concentrations indicate that affinity is unchanged but the number of sites appears to increase (*9*).

To determine whether tolerance and physical dependence are related to alterations in receptor binding, we assayed receptor binding in mice implanted with morphine pellets (Table 1), which produce peak tolerance and physical dependence at 3 days (*10*). Between 2 and 106 hours after pellet implantation, receptor binding is enhanced to a similar extent as in injected mice; binding returns to control levels at 156 hours, when the pellets no longer release morphine (*10*). Since tolerance and physical dependence in these mice develop gradually, with a maximal fivefold increase in tolerance to morphine at 60 hours, the enhanced receptor binding appears unrelated to tolerance and physical dependence (*10*).

We evaluated the possibility that enhanced receptor binding is related to generalized behavioral excitation or depression by measuring [^{3}H]dihydromorphine binding in the brains of mice treated with large doses of reserpine (15 mg/kg daily) for 3 days, sodium pentobarbital (100 mg/kg) for 12 hours, or *d*-amphetamine for 2 days (10 mg/kg on day 1 and 5 mg/kg twice on day 2). None of these treatments alters specific receptor binding.

It has been suggested that opiate antagonists enter the brain more readily than opiate agonists (*11*). However, these differences are insufficient to account for the large differences in potency described here.

We reported that receptor binding of [^{3}H]naloxone, an opiate antagonist, was not reduced by sodium (*2*), while Simon *et al.* (*5*) showed that the binding of the agonist [^{3}H]etorphine was decreased by sodium and potassium; these results suggest possible differential effects of "ionic strength" on agonist and antagonist binding. Incubation with 100 m*M* NaCl reduces the binding of the opiate agonists [^{3}H]levorphanol, [^{3}H]oxymorphone, and [^{3}H]-dihydromorphine by 30 to 70 percent (Table 2). By contrast, binding of the antagonists [^{3}H]levallorphan, [^{3}H]naloxone, and [^{3}H]nalorphine is increased 30 to 140 percent. As little as

Table 2. Effects of sodium on binding of ^{3}H-labeled opiate agonists and antagonists to rat brain homogenates. Stereospecific binding of tritiated opiates was determined by incubating 1.9-ml portions of washed rat brain homogenate (Table 3) in the presence of 100 n*M* (−)-3-hydroxy-*N*-allylmorphinan (levallorphan) or 100 n*M* (+)-3-hydroxy-*N*-allylmorphinan. Concentrations of labeled opiates were 1 to 40 n*M*. Control incubations in the absence of NaCl were performed in 0.05*M* tris-HCl buffer, *p*H 7.4, at 37°C. Ratios of specific to nonspecific binding ranged from 3 : 1 to 8 : 1. Values represent triplicate determinations from a typical experiment that was replicated three times. All tritiated opiates appear to bind to the same population of opiate receptor sites (*2*, *9*).

^{3}H-labeled drug	Control stereospecific binding (count/min)	Binding in 100 m*M* NaCl (% of control)
Agonists		
Dihydromorphine	2256 ± 113	30
Oxymorphone	669 ± 32	56
Levorphanol	1292 ± 61	72
Antagonists		
Nalorphine	408 ± 22	145
Naloxone	1582 ± 80	241
Levallorphan	2861 ± 123	129

Table 3. Effects of sodium on inhibition by opiate agonists and antagonists of stereospecific [^{3}H]naloxone binding to rat brain homogenates. Inhibition of stereospecific [^{3}H]naloxone binding was determined in the presence and absence of 100 m*M* NaCl for 14 nonradioactive opiates, with (+)- and (−)-3-hydroxy-*N*-allylmorphinan to assess specificity. Rat brain with cerebellum removed was homogenized (Polytron PT-10, 3000 rev/min) in 100 volumes of 0.05*M* tris buffer and centrifuged at 40,000*g* for 10 minutes. After the supernatant fluid (which contains no specific binding) was discarded, the pellet was reconstituted in the original volume of tris buffer. Seven to ten concentrations of each drug were incubated with 1.5 n*M* [^{3}H]naloxone in the presence and absence of 100 m*M* NaCl. The concentration of drug that produced a 50 percent inhibition of control stereospecific binding (ED_{50}) was determined by log probit analysis. Control [^{3}H]naloxone binding values in the presence and absence of 100 m*M* NaCl (0.05*M* tris-HCl buffer, *p*H 7.4, at 37°C) were 1163 ± 104 and 2806 ± 198 counts per minute, respectively, at 44 percent counting efficiency.

Nonradioactive opiate	ED_{50} of stereospecific [^{3}H]naloxone binding (n*M*)		ED_{50} ratio, +NaCl/−NaCl
	No NaCl	100 m*M* NaCl	
Naloxone	1.5	1.5	1.0
Naltrexone	0.5	0.5	1.0
Diprenorphine	0.5	0.5	1.0
Cyclazocine	0.9	1.5	1.7
Levallorphan	1.0	2.0	2.0
Nalorphine	1.5	4	2.7
Pentazocine	15	50	3.3
Etorphine	0.5	6	12
Meperidine	3000	50,000	17
Levorphanol	1.0	15	15
Oxymorphone	1.0	30	30
Dihydromorphine	3	140	47
Propoxyphene	200	12,000	60
Phenazocine	0.6	80	133

0.5 m*M* NaCl markedly enhances [^{3}H]-naloxone binding, and maximal increase occurs at concentrations around 100 m*M*. Double reciprocal analysis indicates that sodium increases the number of binding sites with no change in affinity for naloxone. Decrease of [^{3}H]dihydromorphine binding is significant in 5 m*M* NaCl, while the greatest decrease occurs in 150 m*M* NaCl, the highest concentration examined. Lithium chloride and the sodium salts Na_2HPO_4 and $NaHCO_3$ affect binding like NaCl, while potassium, rubidium, and cesium inhibit binding of both agonists and antagonists (*9*).

It should be possible to use this sodium effect to discriminate opiate receptor binding by nonradioactive agonists and antagonists. In the presence of sodium, opiates of predominantly agonistic nature should have decreased potency in preventing the binding of an isotopically labeled antagonist. By contrast, opiates of predominantly antagonistic nature should have the same capacity to inhibit the binding of antagonists whether sodium is present or absent. We examined the effect of sodium on the capacity of 14 unlabeled opiates to inhibit the specific binding of 1.5 n*M* [^{3}H]naloxone (Table 3).

The pure antagonists naloxone, naltrexone, and diprenorphine displayed no change in affinity for the receptor in the presence of sodium (Table 3). By contrast, for the opiate agonists etorphine, levorphanol, meperidine, oxymorphone, dihydromorphine, propoxyphene, and phenazocine, inhibitory potency decreased by a factor of 12 to 133 in the presence of sodium. The three antagonists "contaminated" by agonist properties, cyclazocine, levallorphan, and nalorphine (*1*), were only slightly affected by sodium, with inhibitory potency decreased by a factor of 1.7 to 2.7 in the presence of sodium. For pentazocine, considered to be a mixed agonist-antagonist opiate (*1*), inhibitory potency decreased by a factor of 3.3, a value intermediate between those of predominantly agonist and antagonist opiates.

Under the low sodium condition reported here and previously used (*2*) in our in vitro system, the opiate agonists morphine, oxymorphone, levorphanol, and etorphine have receptor affinities similar to those of their corresponding structurally analogous antagonist derivatives nalorphine, naloxone, levallorphan, and diprenorphine, respectively. Yet opiate antagonists are pharmacologically active at considerably lower doses than agonists in vivo (*12*), a difference not attributable to drug distribution in vivo. Indeed, a 10- to 1000-fold greater potency of antagonists compared to agonists in enhancing receptor binding in mice is reported here.

At the sodium concentrations that normally prevail in the extracellular space of mammalian organisms, antagonists should bind more efficiently than agonists to opiate receptor sites. Thus, the differential influence of sodium on the receptor binding of opiate agonists and antagonists probably accounts for their quantitatively different potencies in vivo.

The qualitatively different pharmacological properties of opiate agonists and antagonists may be a reflection of the qualitatively different effect of sodium at receptor sites. The ability to quantify the relative opiate agonist and antagonist activity of drugs by a simple biochemical method may have therapeutic potential for developing "pure" and potent antagonists for treatment of heroin addiction as well as mixed agonist-antagonist drugs that might be nonaddicting analgesics.

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