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RECEPTOR BINDING AND PHARMACOLOGICAL ACTIVITY OF OPIATES IN THE GUINEA-PIG INTESTINE[1]

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ABSTRACT

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A comparison was made between the affinities of a wide range of opiate agonists, mixed agonist-antagonists and antagonists for opiate receptor binding sites in the guinea-pig intestine longitudinal muscle and myenteric plexus preparation, and their pharmacological potency in influencing the electrically induced contraction of this *in vitro* functional system. The relative affinities of drugs and the degree of stereospecificity for intestinal binding sites are closely similar to these properties in the brain. Receptor binding correlates extremely well with pharmacological potency, both for agonists and antagonists, indicating that binding involves pharmacologically relevant opiate receptors. Pharmacological activity correlates best with receptor binding assayed in the presence of sodium.

Specific opiate receptor binding in vertebrate central and peripheral neural tissue has been identified (Pert and Snyder, 1973a,b; Simon *et al.*, 1973; Terenius, 1972, 1973). It has been characterized in terms of its regional distribution (Kuhar *et al.*, 1973; Hiller *et al.*, 1973) its localization in synaptic membranes (Pert *et al.*, 1974), sensitivity to enzymatic digestion and protein modifying reagents (Simon *et al.*, 1973; Pasternak and Snyder, 1974; Wilson *et al.*, 1975; Pasternak and Snyder 1975). The influence of opiate addiction has been studied (Pert *et al.*, 1973; Klee and Streaty, 1974; Hitzemann *et al.*, 1974). Such binding has also been found in a neuroblastoma X glioma hybrid cell line (Klee and Nirenberg, 1974). This binding probably involves the pharmacologically relevant opiate receptors because affinities of numerous opiate agonists and antagonists parallel, in general, their analgesic or narcotic antagonist potency (Pert and Snyder, 1973a,b; Terenius, 1974), with the best correlation found when the opiate binding assay is conducted in the presence of physiological concentrations of sodium which enhances the binding of antagonists and reduces that of agonists (Pert *et al.*, 1973; Pert and Snyder, 1974).

In order to relate opiate receptor binding with greater precision to pharmacological activity, both properties should be measured in the same tissue. Moreover, by directly comparing

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receptor binding with pharmacological potency in the same tissue several important issues in receptor theory can be investigated, such as the roles of "intrinsic activity" and "spare receptors" in determining pharmacologic actions.

The ability of opiate agonists to inhibit the electrically induced contractions of the longitudinal muscle of the guinea-pig ileum, or the ability of opiate antagonists to reverse this inhibition, closely parallels their *in vivo* pharmacological potency providing a valuable *in vitro* system to study opiate pharmacology (Paton, 1957; Cox and Weinstock, 1966; Gyang and Kosterlitz, 1966; Kosterlitz and Watt, 1968; Fennessy *et al.*, 1969; Kosterlitz *et al.*, 1973). The site of opiate action is neuronal, within the myenteric plexus, and is mediated by a decrease in the release of acetylcholine following nerve stimulation (Paton, 1957; Cox and Weinstock, 1966; de la Lande and Porter, 1967; Paton and Zar, 1968; Greenberg *et al.*, 1970; Lees *et al.*, 1973). Specific opiate receptor binding in the ileum is also restricted to the myenteric plexus and is not present on the longitudinal muscle itself (Pert and Snyder, 1973a).

In order to explore these issues, we have compared opiate receptor binding in the guinea-pig intestine with opiate effects on its electrically induced contractions.

Methods

Male, albino Cam guinea pigs (300–400g) were killed by decapitation and the small intestine was rapidly removed. The duodenum and the terminal 10 cm of the ileum were discarded and the remaining intestine was placed in Krebs-Ringer–bicarbonate solution (NaCl, 118 mM; KCl, 4.75 mM; $CaCl_2$, 2.54 mM; KH_2PO_4, 1.19 mM; $MgSO_4$, 1.2 mM; $NaHCO_3$, 25 mM; glucose, 11 mM; pH 7.4) which was gassed with 95% O_2–5% CO_2 and maintained at 37°C. The longitudinal muscle with attached myenteric plexus was removed by the method of Ambache (1954) and Rang (1964) and placed in the Krebs' solution.

Opiate binding studies. Muscle strips were placed in ice-cold Krebs-Tris solution (composition as above with the $NaHCO_3$ replaced with Tris-HCl, 25 mM, and KH_2PO_4 and glucose omitted, pH 7.4 at 37°C) for 5 minutes. They were then removed, blotted on filter paper, weighed and returned to a small volume of ice-cold Krebs-Tris solution. The tissue was minced by repeated cutting with a small pair of scissors and then homogenized with a Polytron (speed 10, 2 minutes). The homogenate was made up to 100 volumes (w/v) with Krebs-Tris solution and centrifuged for 20 minutes (49,000 × *g*, 0°C), the supernatant fluid was discarded and the pellets were resuspended in the original volume of ice-cold Krebs-Tris solution.

Saturation curves of specific ^{3}H-naloxone and ^{3}H-dihydromorphine binding were generated by incubating 2-ml aliquots of homogenate (20 mg of wet weight tissue) with increasing concentrations of the labeled ligand in the presence and absence of 2 μM (−)-3-hydroxy-N-allyl-morphinan (levallorphan) in triplicates at 37°C for 20 minutes. After incubation the samples were immediately filtered under reduced pressure without cooling through Whatman glass fiber filters (GF-B), which were then washed with two 5-ml portions of ice-cold Krebs-Tris solution. The filters were extracted in Hydromix solution (Yorktown Research, New York, N.Y.) overnight, and radioactivity was determined by liquid scintillation spectrometry, at a counting efficiency of 47%. Specific receptor binding of the labeled ligand was determined by subtracting binding of the labeled ligand in the presence of 2 μM levallorphan from binding in its absence, at each concentration of the labeled ligand (Pasternak and Snyder, 1974). Saturation data for ^{3}H-naloxone binding was also obtained for a mince preparation, which was not homogenized. Two-milliliter aliquots of the mince (60 mg of wet weight tissue) in Krebs-Ringer–bicarbonate solution (pH 7.4) were incubated in 20-ml beakers at 37°C for 20 minutes in a Dubnoff metabolic shaking incubator which was gassed with 95% O_2–5% CO_2. The samples were filtered as above and the filters extracted in 1 ml of Protosol (New England Nuclear Corp., Boston, Mass.) at 37°C overnight and counted in Aquasol (New England Nuclear).

The mince preparation in Krebs-Ringer–bicarbonate solution most closely approximates the conditions under which the pharmacological properties of the opiates in inhibiting the electrically induced contractions of the strips were measured. Because minces and homogenates provided identical calculated K_D values for naloxone, further opiate binding assays employed homogenates which gave greater reproducibility and utilized less tissue per determination. Analysis of ^{3}H-dihydromorphine saturation in rat brain homogenates used the same conditions as experiments with intestinal homogenates. All incubations with ^{3}H-dihydromorphine were performed in the dark because of the photosensitivity of this compound.

Determinations of EC50 values. Two milliliter aliquots of homogenate were preincubated at 37°C for 10 minutes in triplicate with six concen-

trations of the unlabeled opiate under study, along with tubes to which either no unlabeled opiate was added or which contained 2 μM levallorphan. After cooling on ice for 5 minutes, 0.68 nM ^{3}H-naloxone was added to each tube, which was shaken in a Vortex mixer and incubated at 37°C for 20 minutes. After incubation, the samples were filtered and counted as above. The concentration of the unlabeled opiate which inhibited the specific ^{3}H-naloxone binding by 50% (EC50) was determined by log-probit analysis. EC50 values for each opiate were determined twice and the mean value was reported.

Determinations of opiate action on the electrically induced contractions of the guinea-pig intestine. The longitudinal muscle strips with attached myenteric plexus were prepared as described above and maintained in Krebs-Ringer-bicarbonate solution (pH 7.4) which was bubbled with 95% O_2–5% CO_2 and maintained at 37°C. For the pharmacological experiments, the Krebs' solution also contained choline chloride (20 μM) and mepyramine maleate (0.125 μM). A strip was attached *via* silk threads in an organ bath (8 ml) to a force-displacement transducer (Grass FT.03C) with the resting tension adjusted to 0.3 g. The organ bath temperature was kept constant by a water circulating jacket at 37°C. The strip was stimulated by supramaximal electrical stimulation *via* two platinum ring electrodes at the top and bottom of the bath (Grass S4E stimulator, 0.1 Hz pulse duration, 1.5 msec) and the twitch-like contractions were displayed on a Grass oscillograph. The strip was allowed to equilibrate under continuous stimulation with washes every 5 to 10 minutes until a constant base-line response was obtained. Opiate agonists were added to the bath during stimulation in volumes of 20 to 40 μl and were allowed to remain in contact with the strips until maximal inhibition of contraction had occurred, an interval which varied among different opiates. The strips showed the most rapid onset of inhibition of contraction with normorphine and recovered to base-line levels fastest after its removal by washing. Dose-response curves for inhibition of contraction were constructed for normorphine (with a 10-minute interval between each drug addition), dihydromorphine and morphine (with a 30-minute interval between each drug addition) and the concentration of drug which inhibited the electrically induced contractions by 50% (ID50) was determined. The ID50 values for inhibition of contraction for other opiate agonists and mixed agonist-antagonists were determined by the "single dose" method of Kosterlitz and Watt (1968), with a normorphine dose-response curve as standard which was constructed in the same strip immediately prior to determination of the ID50 of the opiate under investigation. Antagonism of the agonist action of normorphine was also investigated by the method of Kosterlitz and Watt (1968). After the construction of a dose-response curve to normorphine, the antagonist under investigation was introduced into the organ bath and remained in contact with the strip for 20 minutes before the addition of the test dose of normorphine. The dose ratio, that is, the ratio of the concentrations of normorphine required to inhibit contractions to the same extent in the presence or absence of the antagonist, was determined and the concentration of antagonist required for an apparent 50% occupancy of receptors (K_e) was then calculated. A separate strip was used for the determination of the above parameters for each opiate under investigation.

Drugs. Drugs were donated by the following companies: Endo Laboratories, Garden City, N.Y. (naloxone, oxymorphone, naltrexone); Ciba-Geigy, Summit, N.J. [6-methano-3-benzazocin-8-ol methanesulfonate (GPA 2163)]; Eli Lilly & Company, Indianapolis, Ind. [(±)-propoxyphene; (+)- and (−)-methadone]; Hoffmann-La Roche, Nutley, N.J. [levorphanol, dextrorphan, levallorphan, (+)-3-hydroxy-N-allylmorphinan]; American Cyanamid, Princeton, N.J. (etorphine, diprenorphine); and Sterling-Winthrop, Rensselaer, N.Y. (meperidine, cyclazocine, pentazocine). Nalorphine was purchased from the Merck Chemical Company, Rahway, N.J. and (−)-phenazocine and normorphine were generously provided by Dr. E. L. May. ^{3}H-naloxone (23.6 c/mmol) and ^{3}H-dihydromorphine (55 c/mmol) were purchased from the New England Nuclear. All drugs are (−)-isomers unless otherwise indicated.

^{3}H-levorphanol (5.4 c/mmol) and ^{3}H-levallorphan (7.5 c/mmol) were prepared at New England Nuclear by catalytic tritium exchange. In our laboratory, the tritiated compounds were purified by thin-layer chromatography and their specific activities were determined by comparison with the ultraviolet absorption of standard solutions (Pert *et al.*, 1973).

Results

Characteristics of opiate receptor binding in the guinea-pig intestine. The properties of ^{3}H-naloxone binding in homogenates of the guinea-pig intestine are very similar to those described previously in rat brain homogenates (Pert and Snyder, 1973a,b) (table 1; fig. 1). Binding is highly stereospecific; levorotatory isomers are much more potent inhibitors

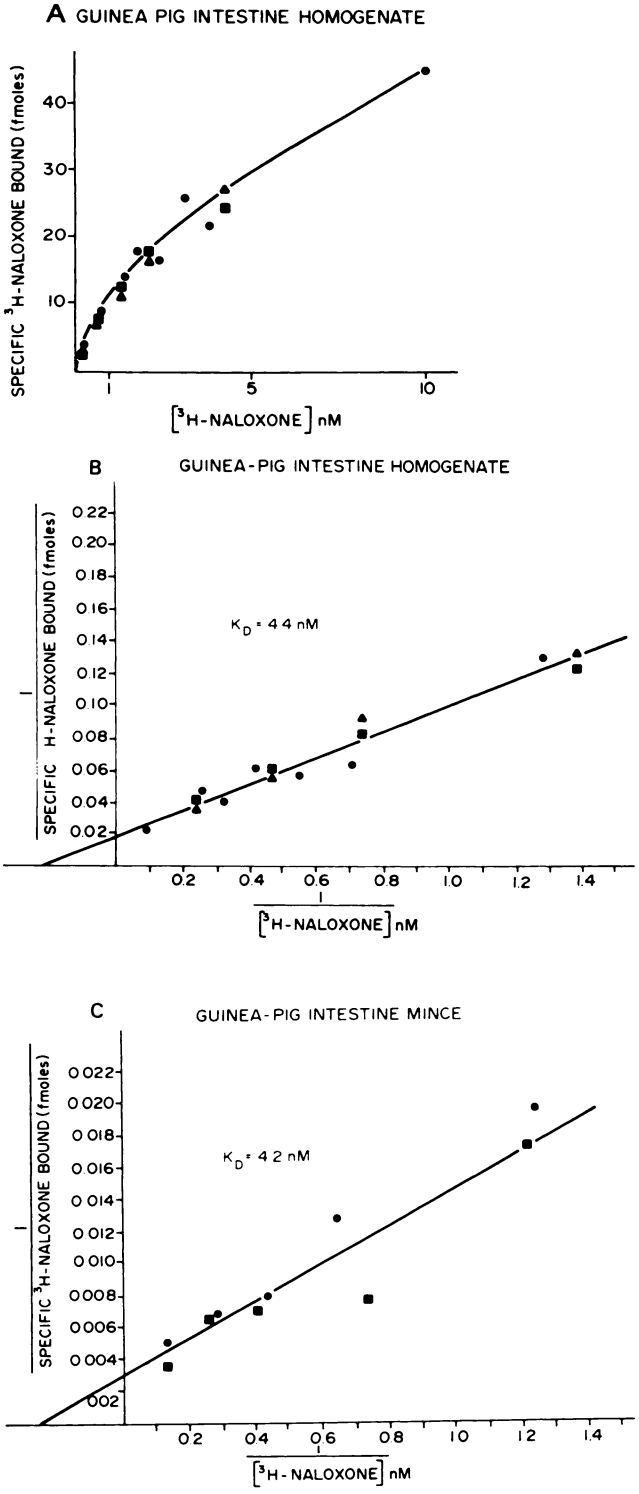

A GUINEA PIG INTESTINE HOMOGENATE
SPECIFIC ³H-NALOXONE BOUND (fmoles)
40
30
20
10
1
5
10
[³H-NALOXONE] nM
B
GUINEA-PIG INTESTINE HOMOGENATE
1 / SPECIFIC ³H-NALOXONE BOUND (fmoles)
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
K_D = 4.4 nM
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1 / [³H-NALOXONE] nM
C
GUINEA-PIG INTESTINE MINCE
1 / SPECIFIC ³H-NALOXONE BOUND (fmoles)
0.022
0.020
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
002
K_D = 4.2 nM
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1 / [³H-NALOXONE] nM

FIG. 1

TABLE 1

Stereospecificity of opiate binding to homogenates of guinea-pig intestine

Opiate	EC50 for Inhibition of Stereospecific ^{3}H-Naloxone Binding
	nM
(−)-3-Hydroxy-N-allylmorphinan (levallorphan)	2.9
(+)-3-Hydroxy-N-allylmorphinan (dextrallorphan)	2800
Levorphanol	30
Dextrorphan	8500
(−)-Methadone	180
(+)-Methadone	2200

Two-milliliter aliquots of homogenates of the guinea-pig intestine longitudinal muscle and myenteric plexus preparation in Krebs-Tris solution were incubated with six concentrations of each drug in the presence of 0.68 nM ^{3}H-naloxone for 20 minutes at 37°C. The concentration of drug that produced 50% inhibition of control stereospecific ^{3}H-naloxone binding (EC50) was determined by log probit analysis.

of ^{3}H-naloxone binding than dextrorotatory isomers. The EC50 for levallorphan, about 2.9 nM, is 1000 times lower than that of the (+)-isomer dextrallorphan. The EC50 for levorphanol, about 30 nM, is 300 times lower than that of dextrorphan. The lesser stereospecificity of levorphanol as compared to levallorphan may simply reflect the reduced stereospecificity of drugs that are relatively less potent (Pfeiffer, 1956). We have shown previously that, in the rat brain, the ability of opiate agonists to displace ^{3}H-naloxone diminishes in the presence of physiological concentrations of sodium while that of antagonists is unaffected (Pert *et al.*, 1973; Pert and Snyder, 1974). Thus, while levorphanol, the agonist corresponding to the antagonist levallorphan, had the same potency as levallorphan in brain homogenates assayed in the absence of sodium, in the presence of sodium it is consideraly less potent than levallorphan. The EC50 for levorphal is 10 times greater than for levallorphan in intestinal homogenates assayed in Krebs-Tris which contains physiological concentrations of sodium. Stereospecificity is much less for the isomers of methadone than that for the other opiates. The two isomers differ by a factor of about 10 in their EC50 value for inhibiting ^{3}H-naloxone binding in intestine homogenates, similar to previous findings with brain homogenates (Pert and Snyder, 1973b). The lesser stereospecificity of the methadone isomers may be related to the greater conformational mobility of the methadone molecule as compared to levorphanol.

Since the pharmacological experiments in this study employed strips on the longitudinal muscle and myenteric plexus, we compared the binding of ^{3}H-naloxone to minces of such strips which contain intact pieces of tissue, with its binding to homogenates of the strips (fig. 1). ^{3}H-naloxone binding is saturable in both homogenates and minces with the same K_D value of about 4 nM for both preparations.

Comparison of opiate receptor binding in homogenates of guinea-pig intestine and rat brain. There is a highly significant correlation between the relative potencies of an extensive series of opiate agonists, mixed agonist-antagonists and antagonists in their ability to inhibit ^{3}H-naloxone binding to homogenates of guinea-pig intestine and to homogenates of rat brain when assayed in the presence of sodium (fig. 2; table 2). A linear regression analysis of the relative potencies of opiates (morphine = 1 in each case) in the intestine and brain receptor binding assays yields a slope of 1.10 which is not significantly different from 1.0 ($P > .05$). The correlation coefficient between the relative potencies of the opiates in the brain and intestine is highly significant ($r = 0.96$, $P < .001$). This close correlation is even

FIG. 1. Binding of ^{3}H-naloxone to homogenates and minces of guinea-pig intestine. A. Saturation of stereospecific ^{3}H-naloxone binding in homogenates of guinea-pig intestine longitudinal muscle and myenteric plexus preparation. Symbols represent the mean results of three separate determinations which varied less than 10%. B. Double reciprocal plot of the saturation of stereospecific ^{3}H-naloxone binding to homogenates of the guinea-pig intestine longitudinal muscle and myenteric plexus preparation. Symbols represent the mean results of three separate experiments. C. Double reciprocal plot of the saturation of ^{3}H-naloxone binding to minces of the guinea-pig intestine longitudinal muscle and myenteric plexus preparation. Symbols represent the mean results of two separate experiments.

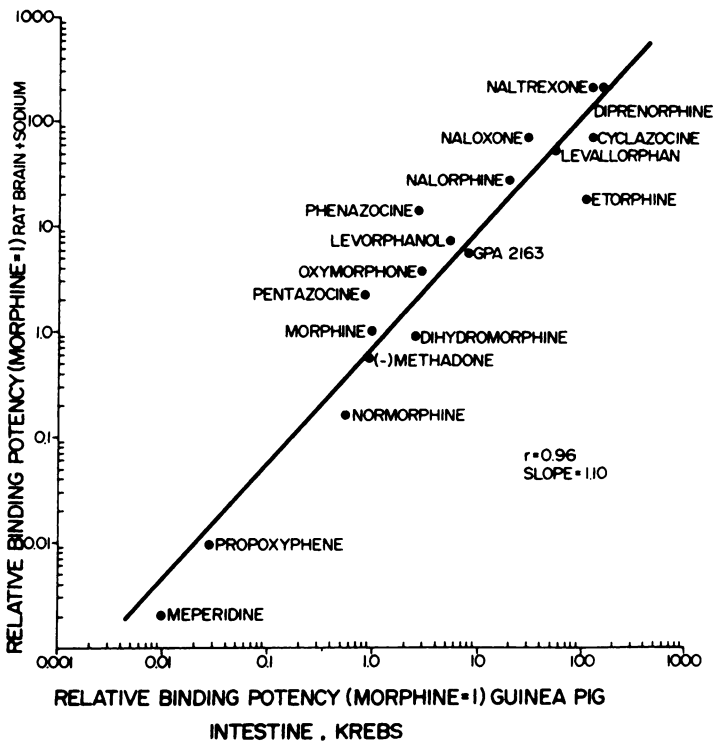


FIG. 2. Correlation between the relative potencies of opiates to inhibit ^{3}H-naloxone binding in guinea-pig intestine and rat brain homogenates. The relative potencies of opiates were calculated from EC50 determinations for each drug to inhibit stereospecific ^{3}H-naloxone binding in either homogenates of the guinea-pig intestine longitudinal muscle and myenteric plexus preparation (assayed in Krebs-Tris, 37°C, pH 7.4) or homogenates of rat brain (assayed in 0.05 M Tris + 100 mM NaCl, 25°C, pH 7.7). The EC50 for morphine was taken as the standard in each system and given the value of 1. The rat brain data are taken from Pert and Snyder (1974). The EC50 values for the intestine are the means of two separate determinations.

more impressive in light of the fact that the assay conditions are somewhat different for the intestine and brain. In order to assay opiate receptor binding in the intestine under conditions similar to those used to evaluate the influence of the opiates in intestinal contractions, binding assays in the intestine were performed in Krebs-Tris solution (pH 7.4) at 37°C, while brain assays had been performed in 0.05 M Tris-HCl (pH 7.7) supplemented with 100 mM sodium chloride at 25°C. Despite these considerations, even the absolute EC50 values in the guinea-pig intestine homogenates closely resemble those of brain homogenates assayed with sodium (table 2). The largest discrepancies involve the two benzomorphan drugs, phenazocine and pentazocine, whose EC50 values respectively are 7- and 4-fold greater in the intestine than in the brain with sodium. Etorphine displays an EC50 in the intestine about one-fourth of its value in the brain with sodium. For most other opiates, discrepancies between the EC50 values in the brain with sodium and intestine are no more than 2- to 3-fold and in many cases the values are virtually identical. Since intestinal assays were performed in the presence of sodium, the EC50 values, especially for agonists, correlate better with values for brain assayed with, rather than without, sodium.

Comparison of the effects of opiates on receptor binding and electrically induced contractions of the guinea-pig intestine. Normorphine is a useful drug for evaluating opiate effects on electrically induced contractions of the guinea-pig intestinal strips because

TABLE 2

EC50 values for the inhibition of ^{3}H-naloxone binding by opiates in homogenates of guinea-pig intestine and rat brain

Opiate	EC50 for Inhibition of Stereospecific ^{3}H-Naloxone Binding		
	Guinea-pig intestine (Krebs-Tris)	Rat brain (Tris + 100 mM NaCl)	Rat brain (Tris, no NaCl)
	nM		
Diprenorphine	0.97	0.5	0.5
Cyclazocine	1.25	1.5	0.9
Naltrexone	1.25	0.5	0.5
Etorphine	1.4	6.0	0.5
Levallorphan	2.9	2.0	1.0
Naloxone	5.3	1.5	1.5
Nalorphine	8.0	4.0	1.5
GPA 2163	23	20	100
Levorphanol	30	15	1.0
Oxymorphone	57	30	1.0
Phenazocine	60	8.0	0.6
Dihydromorphine	65	140	3.0
Morphine	170	110	3.0
(−)-Methadone	180	200	7.0
Pentazocine	200	50	15
Normorphine	300	700	15
Propoxyphene	6,000	12,000	200
Merperidine	17,500	50,000	3,000

Six to 10 concentrations of each unlabeled opiate were incubated with homogenates of the guinea-pig intestine longitudinal muscle and myenteric plexus preparation or rat brain in the presence of ^{3}H-naloxone. The concentration of drug that produced 50% inhibition of control stereospecific ^{3}H-naloxone binding (EC50) was determined by log probit analysis and values presented are the means of two or three determinations. Guinea-pig intestine was homogenized in Krebs-Tris solution and assayed at 37°C (pH 7.4) while rat brain was homogenized in 0.05 M Tris and assayed at 25°C (pH 7.7) ±100 mM NaCl. The rat brain data are taken from Pert and Snyder (1974). Linear regression analysis (fig. 2) reveals a slope of 1.10 and correlation of 0.96 between the relative EC50 values in intestine and brain assayed with sodium and a slope of 0.70 and correlation of 0.82 between the relative EC50 values for intestine compared to brain assayed in the absence of sodium. The slopes for comparison of intestine with brain in the presence or absence of sodium differ significantly ($P < .05$).

of its quick onset of action and the rapid recovery of contractions when the normorphine is washed out. Thus the period before maximal effects are obtained with normorphine is 1 minute, the corresponding interval for dihydromorphine is 6 minutes, for etorphine, about 10 minutes and for codeine, 15 minutes (fig. 3). Similarly, intestinal contractions recover to control values within 5 minutes after normorphine is washed out as compared to 15 minutes for dihydromorphine. Accordingly, a full dose-response for normorphine can be generated in a relatively short period of time over which there is no decline in the control level of contractions or any change in the sensitivity of the preparation. These observations confirm the extensive

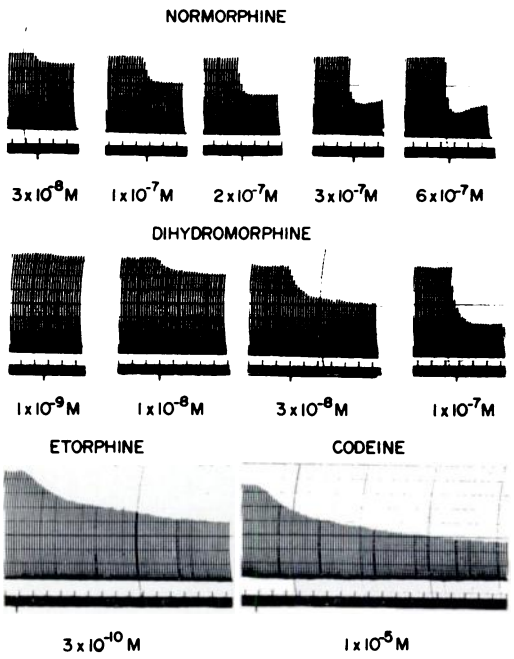


FIG. 3. Inhibition of the electrically induced contractions of the guinea-pig intestine by opiates. The inhibition of the electrically induced contractions of the guinea-pig intestine longitudinal muscle and myenteric plexus preparation by normorphine, dihydromorphine, etorphine and codeine are shown. The upper trace in each case represents the degree of contraction of the muscle which was supramaximally stimulated every 10 seconds for 1.5 msec. The lower trace is a time scale with the deflections upwards representing 1-minute intervals. The deflection downwards indicates the addition of the drug under study. A separate muscle strip was used for each of the different drugs under study.

and elegant studies of Kosterlitz and his collaborators (Kosterlitz *et al.*, 1973).

The potency of normorphine in inhibiting the electrically induced contractions of the intestinal strips correlates closely with its potency in inhibiting ^{3}H-naloxone binding (fig. 4). Indeed, at all concentrations of normorphine examined the extent of inhibition of ^{3}H-naloxone binding and of the electrically induced contractions is essentially the same. The slopes of the dose-response curves generated for normorphine in the binding assay and in the pharmacological assay are closely similar and the EC50 for both processes are the same, about 250 nM.

To compare in greater detail the correlation between potency for receptor binding and for pharmacological actions, we evaluated a wide range of opiate agonists and antagonists, determining ID50 values for agonist activity corresponding to the molar concentration for a 50% maximal pharmacological response and K_e values for antagonist activity. The EC50 values for opiate receptor binding were transformed into apparent K_D values by correcting for the small fraction of opiate sites occupied by the ^{3}H-naloxone in the assay system using the Langmuir binding equations (Colquhoun, 1973). This transformation involves dividing the EC50 value by 1.15, a factor determined by the 0.68 nM concentration of ^{3}H-naloxone and the K_D for naloxone. The resultant K_D values presumably represent the concentrations of the various drugs required to occupy 50% of receptor binding sites and were compared with the ID50 concentrations of these drugs to inhibit the electrically induced contractions of the guinea-pig intestinal strip by 50% or with the K_e concentration for antagonists (fig. 5).

There is an extremely close correlation between the effects of drugs on ^{3}H-naloxone binding and on electrically induced contractions. The extent of the correlation between opiate effects on binding and on contraction appears to be the same for pure agonists, mixed agonist-antagonists and for relatively pure antagonists. The linear regression analysis for these values for all drugs describes a line with a slope of 0.98 which does not differ significantly from 1.0, representing a perfect correlation. The extent of the correlation is statistically impressive ($r = 0.97$, $P < .001$) (fig. 5).

In our initial studies of opiate receptor binding in the rat brain, we found evidence for only one saturable component of opiate binding (Pert and Snyder, 1973b). More recently, using opiates of much higher specific activity, it has been possible to demonstrate a novel higher affinity binding site in addition to the initially described sites of somewhat lower affinity (Pasternak and Snyder, 1975). In guinea-pig intestine homogenates as well as in rat brain homogenates assayed in Krebs-Tris solution at 37°C, there is evidence for two saturable components of ^{3}H-dihydromorphine binding (fig. 6). The high-affinity site in both tissues has a K_D of about 0.9 nM, while the K_D for the low-affinity site is 25 nM in rat brain and 65 nM in intestinal homogenates. The significance of this apparent 2.6-fold difference in K_D values for guinea-pig intestine and rat brain is unclear. Since the concentration of dihydromorphine which inhibits electrically induced contractions of the intestine by 50% is 62 nM, the low-affinity binding sites for dihydromorphine in the intestine may be the pharmacologically relevant ones. However, if high- and low-affinity binding represents opiate interactions with two interconvertible receptor conformations (Pasternak and Snyder, 1975), other interpretations may be appropriate (see "Discussion").

Similar experiments were performed for ^{3}H-levorphanol binding. High- and low-affinity binding components for ^{3}H-levorphanol have respective K_D values of about 1 and about 20 nM. The concentration of levorphanol which inhibits electrically induced contractions of the intestinal strips is 30 nM, corresponding to the K_D value for the low affinity binding site. Interestingly, in the guinea-pig intestinal homogenates, we do not detect evidence for multiple binding sites for ^{3}H-naloxone (fig. 1) as observed in the brain (Pasternak and Snyder, 1975). Moreover, with ^{3}H-levallorphan we observe only one component of receptor binding with a K_D value of 2.9 nM.

The calculated total number of low-affinity binding sites for ^{3}H-dihydromorphine in guinea-pig intestinal homogenates is 55 fmol/mg of protein. The corresponding value for low-affinity binding sites for ^{3}H-levorphanol in guinea-pig intestinal homogenates is 62 fmol/mg of protein. For ^{3}H-levallorphan and ^{3}H-naloxone

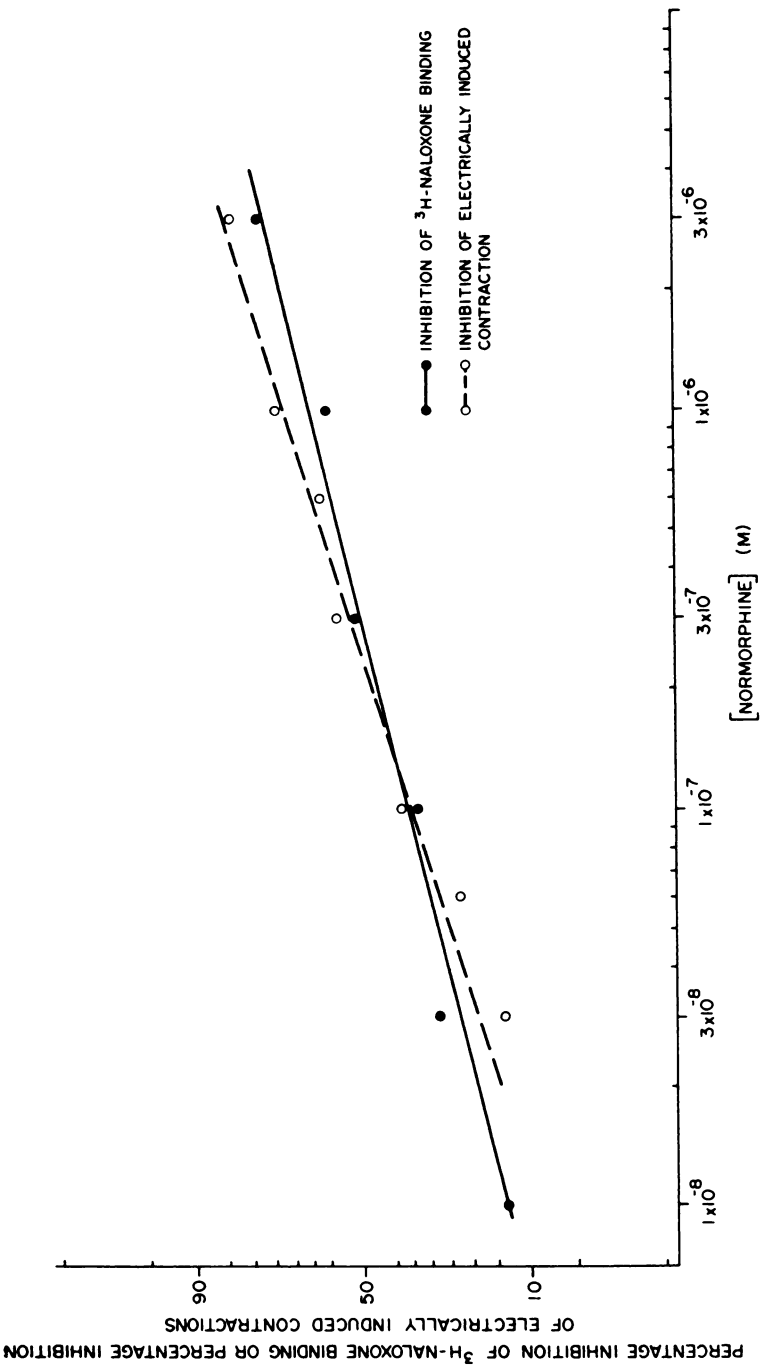


FIG. 4. Correlation between the pharmacological activity and receptor binding of normorphine. The percent inhibition of the electrically induced contractions of the guinea-pig intestine longitudinal muscle and myenteric plexus preparation and the percent inhibition of stereospecific ^{3}H-naloxone binding in homogenates of the guinea-pig intestine longitudinal muscle and myenteric plexus preparation are plotted as a function of normorphine concentration on log probit paper.

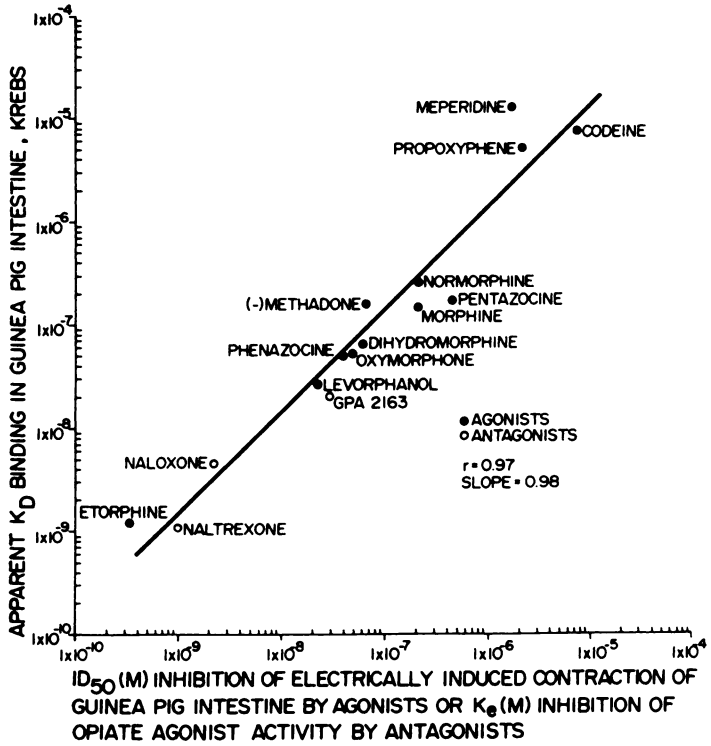


FIG. 5. Correlation between receptor binding and pharmacological activities of opiates in the guinea-pig intestine. The K_D values (determined by the inhibition of stereospecific ^{3}H-naloxone binding in homogenates of the guinea-pig intestine longitudinal muscle and myenteric plexus preparation) for a series of opiate agonists and antagonists are plotted against the ID50 concentration, for agonists, required to inhibit the electrically induced contractions of the guinea-pig intestine longitudinal muscle and myenteric plexus preparation by 50%, or the K_e value for antagonist inhibition of agonist activity in the same preparation.

the total number of sites are 62 and 29 fmol/mg protein respectively. Thus, the total number of receptor binding sites is essentially the same for dihydromorphine, levorphanol and levallorphan. The significance of the lower number of calculated sites for ^{3}H-naloxone is unclear and could merely be a technical artifact related to the greater difficulty of receptor assay in intestine than brain. For the brain, with numerous ligands, the total number of receptors is a constant value (Pert and Snyder, 1973b, 1974; Simon *et al.*, 1973)

Discussion

A major finding of the present study is the extraordinarily close correlation between the affinity for opiate receptor binding sites and the ability of opiate agonists and antagonists to influence electrically induced contractions of the guinea-pig intestine. These data provide strong evidence that the receptor binding sites investigated here and in our previous studies are those which are "pharmacologically relevant."

The detailed comparison of binding and pharmacological potencies in the present study may have bearing on certain questions regarding the mechanisms of drug-receptor interaction. Some receptor theories, based on studies of drug effects on guinea-pig intestinal contractions, posit that for many agonist drugs, a maximal pharmacological response can be elicited when only a small fraction of pharmacologically active receptors are occupied (Stephenson, 1956; Nickerson, 1956; Ariens *et al.*, 1960). However, in these previous studies receptor occupation was never measured directly with a radioactively labeled ligand, but inferred from the pharmacological response. If a sub-

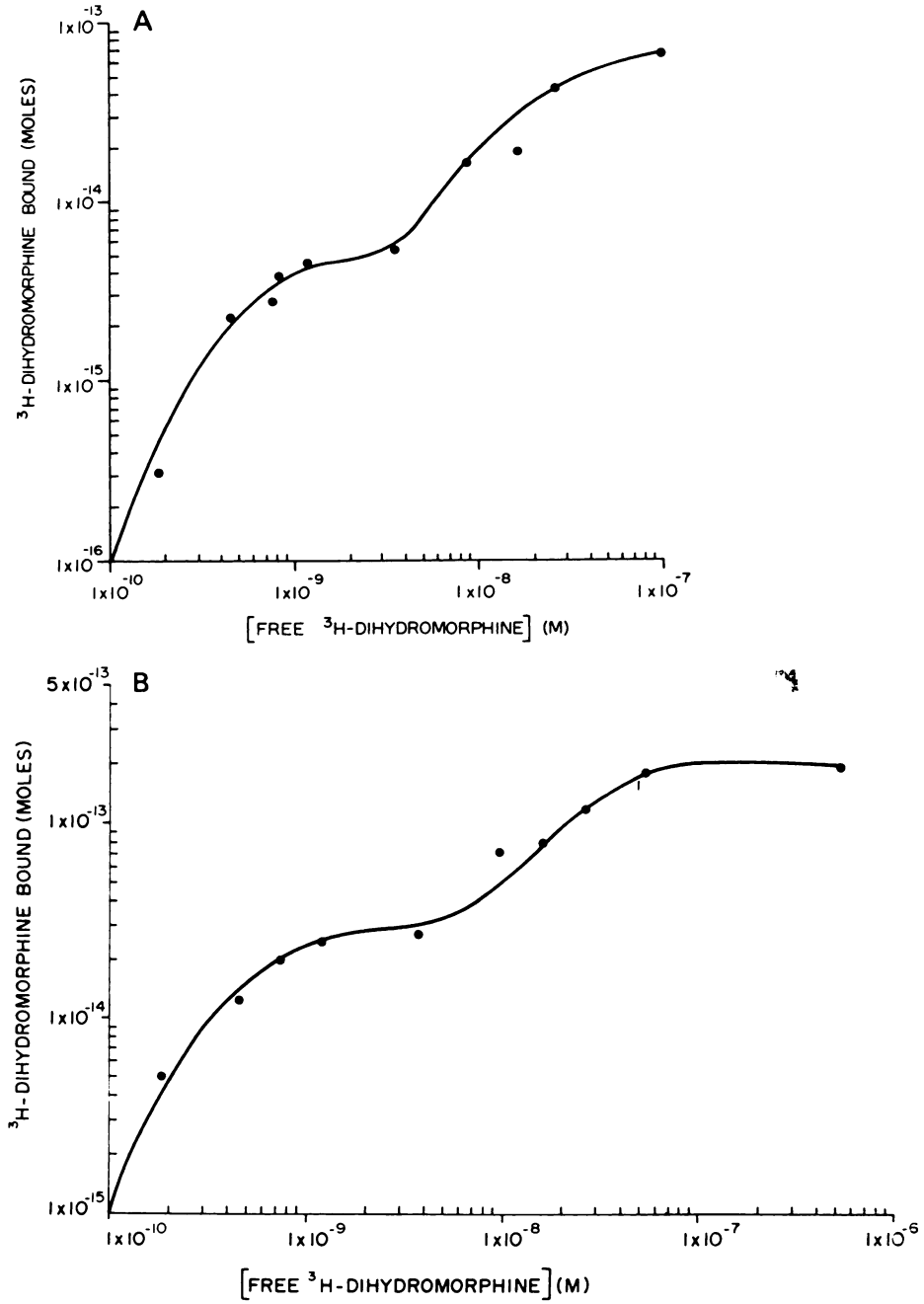
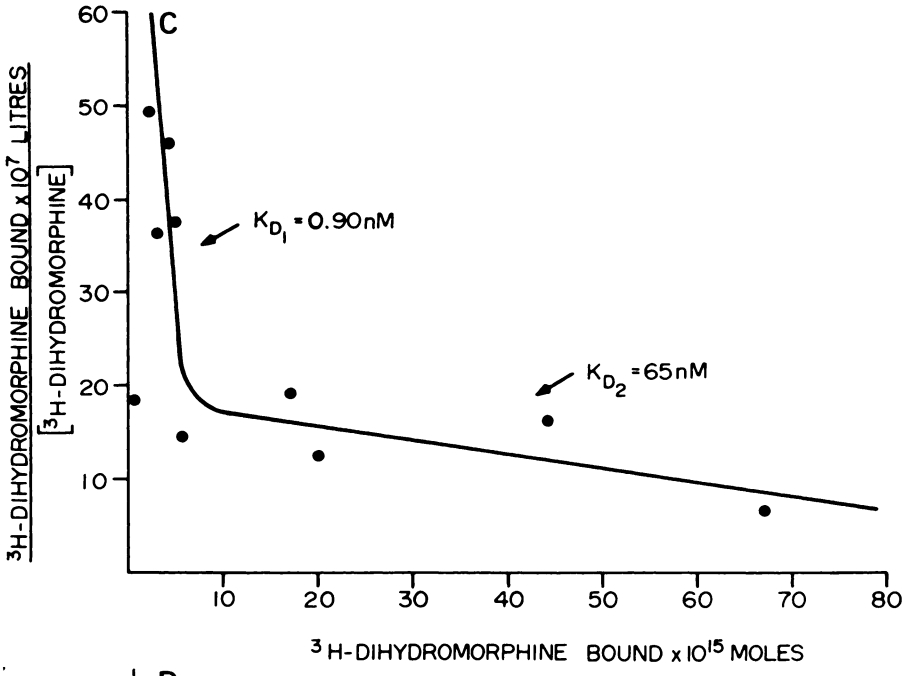


FIG. 6. A to D. Binding of ^{3}H-dihydromorphine in homogenates of guinea-pig intestine and rat brain. A. Saturation of stereospecific ^{3}H-dihydromorphine binding in homogenates of the guinea-pig intestine longitudinal muscle and myenteric plexus preparation assayed in Krebs-Tris at 37°C. B. Saturation of stereospecific ^{3}H-dihydromorphine binding in homogenates of rat brain assayed in Krebs-Tris at 37°C.

FIG. 6. C. Scatchard plot of ^{3}H-dihydromorphine binding in homogenates of the guinea-pig intestine longitudinal muscle and myenteric plexus preparation assayed in Krebs-Tris at 37°C. D. Scatchard plot of ^{3}H-dihydromorphine binding in homogenates of rat brain assayed in Krebs-Tris at 37°C.


C
K_{D_1} = 0.90nM
K_{D_2} = 65nM
^{3}H-DIHYDROMORPHINE BOUND x 10^7 LITRES
[^{3}H-DIHYDROMORPHINE]
^{3}H-DIHYDROMORPHINE BOUND x 10^{15} MOLES

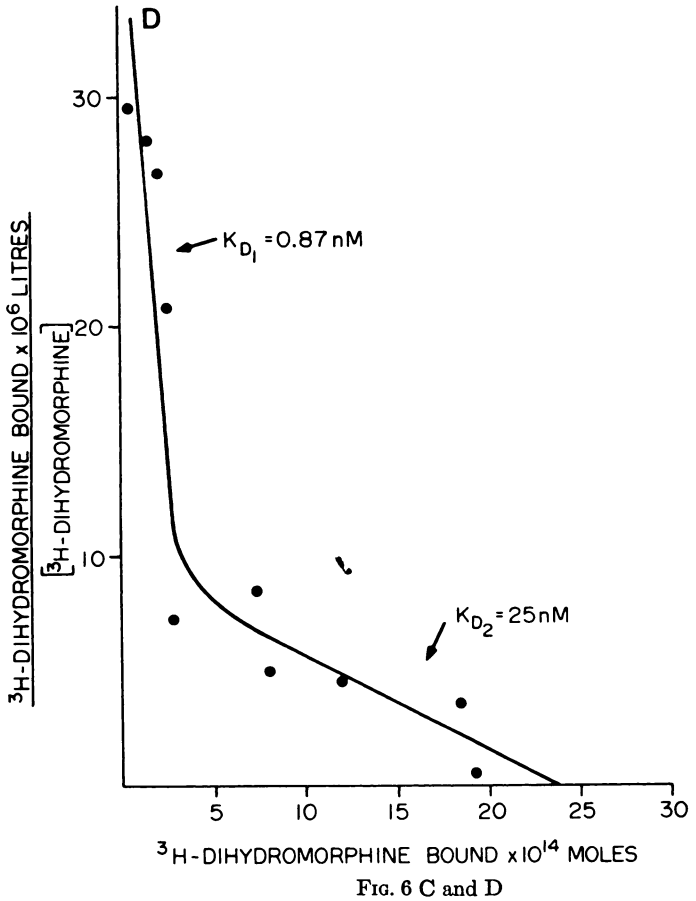

D
K_{D_1} = 0.87nM
K_{D_2} = 25nM
^{3}H-DIHYDROMORPHINE BOUND x 10^6 LITRES
[^{3}H-DIHYDROMORPHINE]
^{3}H-DIHYDROMORPHINE BOUND x 10^{14} MOLES

FIG. 6 C and D

stantial proportion of opiate receptors are "spare receptors," then there should be major differences between the concentration of drugs which occupy 50% of receptor sites as measured in binding studies and the concentrations which produce 50% of the maximal pharmacological response. For four ^{3}H-opiate agonists and antagonists the K_D value, representing the concentration of drug for 50% receptor occupation, was determined directly from the binding of the drug to receptor sites. For a large number of nonradioactive opiate agonists and antagonists, the K_D value was determined from the concentration of the drug which inhibited ^{3}H-naloxone binding by 50%. In all cases, the K_D value for receptor binding corresponds closely to the ID50 or K_e values for pharmacological responses. There is no evidence for major, systematic discrepancies. There is also an excellent agreement between the complete dose-response curves for normorphine in the binding and pharmacological studies. Thus our data suggest that pharmacological responses to opiates in the guinea-pig intestine can be explained without invoking the concept of spare receptors. It is possible that some of the binding demonstrated may correspond to "silent receptors" (Goldstein, 1949) which bind opiates but do not provide a biological effect.

It has been suggested that the many variations in pharmacological potency among drugs are determined by the relative contribution of affinity for receptor sites and "intrinsic activity" (Ariens, 1954; Ariens *et al*, 1957) or "efficacy" (Stephenson, 1956). According to these theories, a series of drugs may all have the same affinity for the receptor but differ in their capability to initiate the biological response, that is, intrinsic activity, and accordingly have different pharmacological potencies. However, the fact that for almost all opiate agonists examined, the concentration required to occupy 50% of receptor binding sites corresponds to the concentration required to produce 50% of the maximal pharmacological response indicates that receptor affinity alone would suffice to account for pharmacological potency of opiate agonist drugs. Similar equilibrium constants for binding and for biological response have been observed also for the muscarinic cholinergic receptor (Paton and Rang, 1965; Yamamura and Snyder, 1974; Snyder *et al.*, 1975), tetrodotoxin binding sites (Colquhoun and Ritchie, 1972) and the insulin receptor (Cuatrecasas, 1971), utilizing radioactive ligands.

One major rationale for invoking the concept of intrinsic activity is the need to explain the actions of pharmacological antagonists. It is usually postulated that an antagonist occupies the agonist receptor site but has no intrinsic activity. While the precise mechanism of action of opiate antagonists is unclear, data from our laboratory on the differential influence of sodium on receptor binding of opiate agonists and antagonists may provide a molecular mechanism accounting for the different intrinsic activity of opiate agonists and antagonists. Sodium enhances the binding of antagonists and diminishes the binding of agonists suggesting that the opiate receptor can exist in two interconvertible allosteric forms (Pert *et al.*, 1973; Pert and Snyder, 1974; Snyder, 1975). In the "sodium" form, the receptor preferentially binds antagonists. In the "non-sodium" form, the receptor binds agonists more efficiently than when the receptor is in the sodium form. If the pharmacologic effects of opiate agonists are mediated by the non-sodium receptors, then by binding to sodium forms of the receptor, opiate antagonists would lower the available number of non-sodium receptors thereby hindering the pharmacological actions of opiate agonists. With normal body sodium levels, the receptor would exist predominantly in the antagonist form. Although agonists can half saturate the non-sodium state of the receptor at low K_D concentrations, the shifting of the equilibrium between the two receptor conformations to obtain sufficient non-sodium receptors to mediate pharmacological agonist responses would require 10 to 100 times greater agonist concentrations. This may explain why relatively high concentrations of agonists, corresponding to the agonist affinity for the sodium receptor state, are required to elicit pharmacological responses.

High- and low-affinity binding may correspond to the two conformations of the opiate receptor (Pasternak and Snyder, 1975). The high-affinity site for antagonists would represent interactions with the sodium-antagonist receptor state, whereas low-affinity antagonist binding (demonstrable in the brain) would represent binding to the agonist conformation. High- and low-affinity binding of agonists

would involve non-sodium and sodium receptor states, respectively.

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