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Opiate Receptor Binding of Agonists and Antagonists Affected Differentially by Sodium

CANDACE B. PERT[1] AND SOLOMON H. SNYDER[2]

Department of Pharmacology and Experimental Therapeutics and Department of Psychiatry and the Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

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SUMMARY

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Receptor binding of the tritiated opiate antagonists naloxone, nalorphine, and levallorphan is enhanced by sodium ion, while binding of the tritiated agonists oxymorphone, dihydromorphine, and levorphanol is diminished. This differential effect of Na^+ is highly specific, since it is elicited by Na^+ and Li^+ but not by other monovalent or divalent cations. The relative effectiveness of nonradioactive opiates in inhibiting [^{3}H]naloxone binding in the absence and presence of Na^+ *in vitro* correlates impressively with their relative agonist-antagonist properties *in vivo*. It is hypothesized that sodium allosterically transforms opiate receptor sites from conformations which bind agonists more readily to conformations which bind antagonists more readily. This hypothesis is supported by the competition of opiate agonists and antagonists for receptor sites, the marked temperature dependence of binding, the similar extent of binding of tritiated agonists and antagonists at maximal saturation, the concurrent increase in naloxone binding sites and decrease in dihydromorphine binding sites caused by the addition of Na^+, and the ability of Na^+ to increase [^{3}H]dihydromorphine dissociation with no effect on [^{3}H]naloxone dissociation.

INTRODUCTION

Although opiate agonists and antagonists are very closely related in chemical structure, they produce dramatically opposite effects. Opiate antagonists specifically and rapidly block or reverse the pharmacological actions of opiate agonists (1). It has been widely assumed that opiate antagonists compete with agonists to occupy the same receptor sites but lack the required "efficacy" (2) or "intrinsic activity" (3) to elicit an effect. Opiate antagonists are much more potent than agonists; agonist effects can be reversed by structurally analogous antagonists in only 0.5–5% of the dose (4–8). The agonist-antagonist properties of opiates appear to form a continuum, since many opiates can be shown to exhibit both agonist and antagonist properties, depending on the behavioral test situation. For example, opiates of the benzomorphan group (9–11) often combine agonist and antagonist activities.

Previously we described the specific binding of tritiated opiate agonists and antagonists to membranes of vertebrate nervous tissue (12–16). This stereospecific binding,

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which was unaffected by high concentrations of non-opiates, was inhibited by a large number of opiates with potencies which closely paralleled their relative pharmacological activities *in vivo* (12, 13). Using this method for studying opiate receptor binding *in vitro*, we undertook a careful comparison of the binding of radiolabeled opiate agonists and antagonists, hoping to identify differences which might be related to their pharmacological actions *in vivo*. We report here that sodium ion specifically enhances the binding of tritiated opiate antagonists while reducing the binding of tritiated opiate agonists. Based upon this differential effect of Na^+, we present a method for estimating the relative agonist-antagonist properties of opiates *in vitro*, and describe studies evaluating the mechanism of the differential effect of Na^+ on receptor binding. A preliminary report of this study has been published (17).

MATERIALS AND METHODS

Drugs were donated by the following companies: Endo, Garden City, N. Y. (naloxone, oxymorphone, naltrexone); Ciba-Geigy [(−)-1,2,3,4,5,6-hexahydro-11β-methyl-6-phenyl-3-propargyl-2,6-methano-3-benzazocin-8-ol methanesulfonate (GPA 2163)]; Lilly, Indianapolis, Ind. [(±)-propoxyphene, (−)-methadone]; Roche, Nutley, N. J. (levorphanol, dextrorphan, levallorphan, (+)-3-hydroxy-*N*-allylmorphinan); American Cyanamid, Princeton, N. J. (etorphine, diprenorphine); and Winthrop, Rensselaer, N. Y. (meperidine, cyclazocine, pentazocine). Nalorphine was purchased from the Merck Chemical Company, Rahway, N. J., and (−)-phenazocine, (−)-etazocine, (−)-*N*-allylnormetazocine, (−)-5-propyl-5-normetazocine, and (−)-metazocine were generously provided by Dr. E. L. May. [^{3}H]Naloxone (23.6 Ci/mmole) and [^{3}H]dihydromorphine (55 Ci/mmole) were purchased from the New England Nuclear Corporation. All drugs are (−)-isomers unless otherwise indicated.

[^{3}H]Oxymorphone (0.8 Ci/mmole), [^{3}H]-levorphanol (5.4 Ci/mmole), [^{3}H]levallorphan (7.5 Ci/mmole), and [^{3}H]nalorphine (3.4 Ci/mmole) were prepared at New England Nuclear Corporation by catalytic tritium exchange (13). 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 (13).

Male Sprague-Dawley rats (175–250 g) were decapitated and their brains were rapidly removed. After removal of the cerebellum, each brain, which weighed 1.5 g, was homogenized in 150 ml of ice-cold 0.05 M Tris-HCl buffer (pH 7.4 at 37°) for 20 sec (Polytron PT-10 homogenizer, 3000 rpm). After centrifugation at 18,000 × *g* for 10 min, the supernatant fluid was discarded and the pellets were reconstituted in the original Tris buffer to obtain the standard "washed" homogenate.

Aliquots of this freshly prepared suspension (1.9 ml) were incubated in triplicate for 30 min at 25° unless otherwise stated. After incubation, samples were transferred to an ice bath, filtered as previously described (12, 13), and washed with two 5-ml portions of ice-cold Tris buffer. Filters were transferred to vials, and after the addition of 12 ml of Hydromix (Yorktown Research, New York) each filter was extracted by vortexing the vial for 10 sec. Vials were counted by liquid scintillation spectrometry at 44% efficiency.

Stereospecific binding was calculated by substracting the binding which occurred in the presence of 100 nM levallorphan from that which occurred in the presence of its pharmacologically inactive enantiomer, 100 nM (+)-3-hydroxy-*N*-allylmorphinan (dextrallorphan). Nonspecific binding in the presence of 100 nM levallorphan was not altered by any ionic manipulations employed in this study. Specific binding represented between 65% (in the case of [^{3}H]nalorphine) and 88% (in the case of [^{3}H]naloxone) of the total binding.

Ions or drugs were always added to the aliquots of homogenates, which were then vortexed briefly before the addition of [^{3}H]-opiate. All incubations were performed in triplicate. [^{3}H]Dihydromorphine binding was always assayed in the dark because of the photosensitivity of this compound.

RESULTS

Influence of sodium ion on binding of tritiated opiate agonists and antagonists to rat brain homogenates. As little as 1 mM sodium chloride enhances [^{3}H]naloxone binding by 60%. Maximal elevation of [^{3}H]naloxone binding occurs at 40–50 mM Na^+, with no marked change up to 200 mM Na^+ (Fig. 1). [^{3}H]Nalorphine and [^{3}H]levallorphan, which are less pure antagonists than naloxone, are less markedly affected by sodium ion, with a constant enhancement of binding by about 30% between 50 mM and 200 mM Na^+. By contrast, binding of the [^{3}H]agonists levorphanol, oxymorphone, and dihydromorphine is consistently depressed by sodium ion. Dihydromorphine appears to be more sensitive to the effects of Na^+ than levorphanol and oxymorphone, with a 25% depression of binding at 1 mM Na^+, a concentration which does not alter the binding of levorphanol or oxymorphone. A nearly maximal 60% depression of dihydromorphine binding occurs at 50 mM sodium ion, with some small further depression at 200 mM. Maximal reduction of [^{3}H]levorphanol and [^{3}H]oxymorphone binding to between 30% and 40% of control values occurs at 100–200 mM Na^+. Other sodium salts, disodium hydrogen phosphate and sodium bicarbonate, enhance [^{3}H]-naloxone binding and reduce [^{3}H]dihydromorphine binding in essentially the same fashion as sodium chloride. The effect of 10, 50, and 100 mM NaCl on [^{3}H]naloxone and [^{3}H]dihydromorphine binding is not significantly different whether 10, 25, or 50 mM Tris buffer is used.

To examine the specificity of the influence of sodium ion on agonist and antagonist binding, we compared the binding of [^{3}H]-naloxone and [^{3}H]dihydromorphine at seven concentrations of Na^+, Li^+, Rb^+, Cs^+ and K^+ between 1 and 150 mM (Fig. 2). Like Na^+, Li^+ also enhances naloxone binding and decreases dihydromorphine binding, but is less effective. Maximal enhancement of [^{3}H]-naloxone binding of 40–50% occurs at 15–150 mM Li^+. The inhibitory effects of Li^+ on [^{3}H]dihydromorphine binding are similar to those of Na^+, although 3 mM Li^+ is required to produce significant inhibition compared to 1 mM in the case of Na^+. Lithium chloride enhances the binding of [^{3}H]levallorphan and depresses the binding of [^{3}H]oxymorphone and [^{3}H]levorphanol (Table 1). By contrast, Rb^+, Cs^+, and K^+ fail to discriminate be-

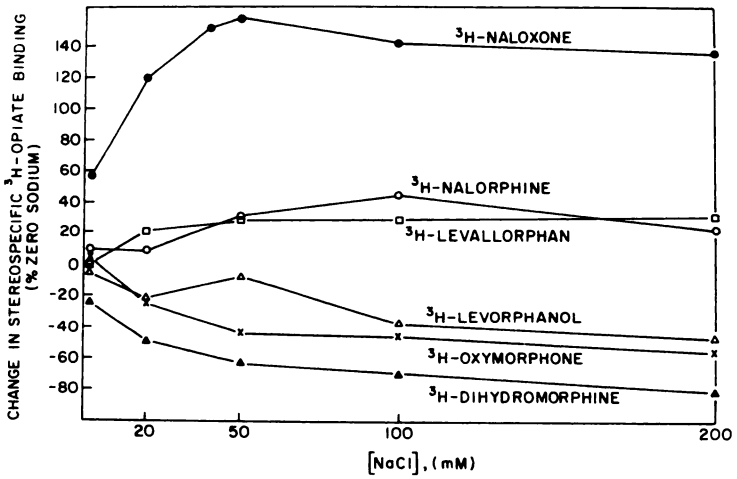

FIG. 1. *Effect of sodium chloride on stereospecific binding of three [^{3}H]opiate agonists and three [^{3}H]opiate antagonists*

Each [^{3}H]opiate was incubated in the presence of levallorphan (100 nM) and dextrallorphan (100 nM) with varied concentrations of sodium chloride and standard rat brain homogenate which had been washed twice. [^{3}H]Naloxone (1 nM), [^{3}H]nalorphine (4 nM), [^{3}H]levallorphan (8.6 nM), [^{3}H]levorphanol (6 nM), [^{3}H]oxymorphone (40 nM), and [^{3}H]dihydromorphine (1 nM), when incubated at 25° for 30 min in the standard binding assay, gave the following stereospecific control (zero sodium) values, respectively: 1292 ± 115 cpm, 355 ± 30 cpm, 2170 ± 195 cpm, 1023 ± 95 cpm, 694 ± 51 cpm, and 2570 ± 141 cpm.

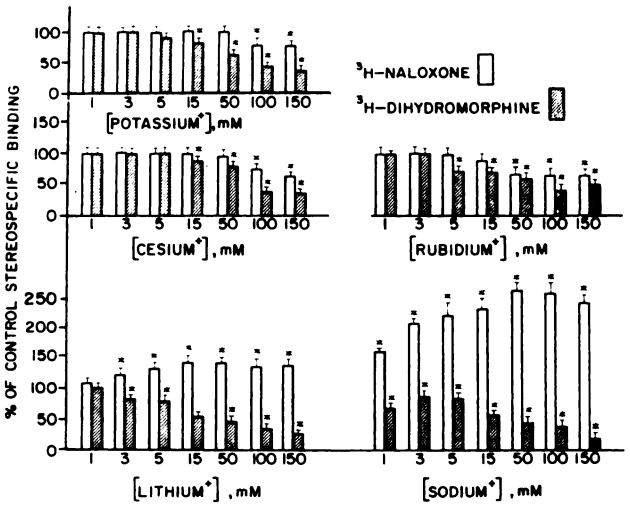

FIG. 2. *Effects of five monovalent cations on stereospecific [³H]naloxone and [³H]dihydromorphine binding*

[³H]Naloxone (1.5 nM) and [³H]dihydromorphine (1 nM) were incubated with 100 nM levorphanol, levallorphan, dextrorphan, or dextrallorphan in the standard binding assay with rat brain homogenate which had been washed once. Seven concentrations (1–150 mM) of each monovalent cation were incubated in triplicate with [³H]naloxone and [³H]dihydromorphine. Control stereospecific binding values were 1096 ± 102 cpm for [³H]naloxone and 2120 ± 201 cpm for [³H]dihydromorphine. Nonspecific binding values, whether determined in the presence of levorphanol or levallorphan, were the same and were unaffected by the cations. Binding values in the presence of dextrallorphan, dextrorphan, or no nonradioactive opiate were not significantly different. Bars denote standard errors of the means.

* $p < 0.005$.

tween naloxone and dihydromorphine, depressing the binding of both drugs at concentrations between 5 and 50 mM, with Rb^+ showing the greatest inhibitory potency. Significant depression of [³H]naloxone binding by these three ions requires a 50 mM concentration, while a maximal depression of 50% occurs between 50 and 150 mM. Dihydromorphine binding is depressed by lower concentrations of Rb^+, Cs^+, and K^+ (5–10 mM) than is naloxone binding. The divalent cations calcium and magnesium depress the binding of [³H]naloxone (17), [³H]-dihydromorphine, [³H]levorphanol, and [³H]-levallorphan, with $CaCl_2$ generally showing greater inhibitory potency (Table 1).

To examine the reversibility of the Na^+ effect, homogenates were incubated at 25° for 5 min in standard Tris buffer in the presence of 10 nM naloxone and 100 mM sodium chloride. The homogenates were centrifuged and the pellet was resuspended and recentrifuged twice in standard Tris-buffer to wash out unbound naloxone and sodium (Fig. 3). This washing procedure, which was sufficient since it fully reversed naloxone inhibition of [³H]naloxone and [³H]dihydromorphine binding, also fully reversed the enhancement of [³H]naloxone binding by Na^+. However, after the washing procedure [³H]dihydromorphine binding was increased by 50% when compared to control preparations initially incubated with Tris buffer in the absence of Na^+ and subjected to the same washing procedure. Thus there appears to be an unexpected "rebound" enhancement of [³H]dihydromorphine binding following the washing procedure, perhaps related to removal of endogenous inhibitors such as cations.

Differential influences of Na^+ interactions of nonradioactive agonists and antagonists with receptors. To evaluate the generality of the sodium ion effects by examining a larger number of opiates, a method for assessing the effect of Na^+ on nonradioactive drugs was developed. The relative ability of nonradioactive opiates to inhibit [³H]naloxone binding in the absence and presence of Na^+ was assessed. Several concentrations of each

nonradioactive opiate were incubated with [^{3}H]naloxone in the absence and presence of 100 mM NaCl. The concentration of drug required to give 50% inhibition of binding was determined by log probit analysis (Fig. 4). Log probit plots of depression of [^{3}H]-naloxone binding by nonradioactive naloxone, nalorphine, and morphine are parallel in both the absence and presence of added Na^+ (Fig. 4). Not surprisingly, the EC_{50} of nonradioactive naloxone is unaltered by the addition of Na^+, since this experiment simply involves an isotope dilution. With the addition of 100 mM Na^+, however, nalorphine and morphine suffer 2.7- and 37-fold losses of inhibitory potency, respectively.

The [^{3}H]naloxone inhibitory potencies for 22 opiates in the absence and presence of 100 mM Na^+ are presented in Table 2, along with their corresponding potencies *in vivo*. The EC_{50} values for the relatively pure antagonists naltrexone (25) and diprenorphine (26) are unaffected by Na^+. The EC_5 value for *N*-allylnormetazocine (27), another rather pure antagonist, is shifted only 1.5-fold by Na^+. However, cyclazocine, levallorphan, and nalorphine, antagonists possessing some agonist properties (28, 29), display a 1.7–2.7-fold sodium shift. By contrast, the predominantly pure opiate agonists (etorphine, phenazocine, levorphanol, meperidine, methadone, oxymorphone, morphine, dihydromorphine, and propoxyphene) manifest very large losses in inhibitory potency, which range from 12- to 60-fold. The benzomorphan mixed agonist-antagonists have unpredictable, highly task-dependent pharmacological properties *in vivo* (Table 2). Drugs of this group (pentazocine, 5-propyl-5-normetazocine, metazocine, and etazocine) show a sodium shift which is intermediate between those of pure agonists and antagonists, with values ranging from 3.3 to 7.1. Interestingly, GPA 2163, a reported pure antagonist which precipitates a long-lasting abstinence syndrome in monkeys (30), inhibits [^{3}H]naloxone binding more potently in the presence than in the absence of sodium ion.

As reported previously (12, 13), the relative potencies of opiate agonists correlate well with pharmacological data when the binding assay is performed in the absence of Na^+ (Table 2). Binding values obtained in the presence of Na^+ correspond better with available pharmacological data. For example, Adler (31) reported that the morphine

TABLE 1

Effects of lithium, potassium, calcium and magnesium ions on stereospecific binding of various[^{3}H]opiates

Aliquots (1.9 ml) of a 100-volume (w/v) Tris-buffered rat brain homogenate were incubated for 30 min at 25° in the presence of levallorphan and dextrallorphan (0.1 μM), various concentrations of added ions, and the stated concentration of [^{3}H]opiate. Control stereospecific binding values with no added ions were 667 ± 16, 1284 ± 38, 1586 ± 71, and 1071 ± 43 cpm for [^{3}H]oxymorphone, [^{3}H]levorphanol, [^{3}H]levallorphan, and [^{3}H]dihydromorphine, respectively. Values represent triplicate determinations, which varied less than 5%. The experiment was replicated twice.

[^{3}H]Opiate	Cation[a]	Concentration	Stereospecific binding
		mM	*% control*
[^{3}H]Oxymorphone, 26 nM	K	100	24
	Li	100	19
[^{3}H]Levorphanol, 8 nM	Ca	5	53
		10	51
	Mg	5	87
		10	62
	K	50	49
		150	25
	Li	15	73
		50	49
		150	22
[^{3}H]Levallorphan, 5 nM	Ca	5	73
		10	64
	Mg	5	89
		10	77
	K	50	67
		150	56
	Li	15	110
		50	131
		150	127
[^{3}H]Dihydromorphine, 0.5 nM	Mg	1	100
		5	82
		10	64
		20	38
	Ca	1	66
		5	56
		10	45
		20	48

[a] All added as the chloride salts.

level in the brains of rats whose analgesic threshold was reduced by half is about 100 nM. Morphine suffers a 37-fold loss of inhibitory potency with the addition of sodium ion, requiring 100 nM concentrations for 50% inhibition in the presence of Na^+. Moreover, at low sodium ion concentration (Table 2) (12, 13), the opiate agonists morphine, oxymorphone, levorphanol, and etorphine have receptor affinities similar, respectively, to those of their corresponding structurally analogous antagonist derivatives, nalorphine, naloxone, levallorphan, and diprenorphine. However, antagonists are much more potent *in vivo* than agonists. The agonists mentioned above suffer a 12–37-fold loss of

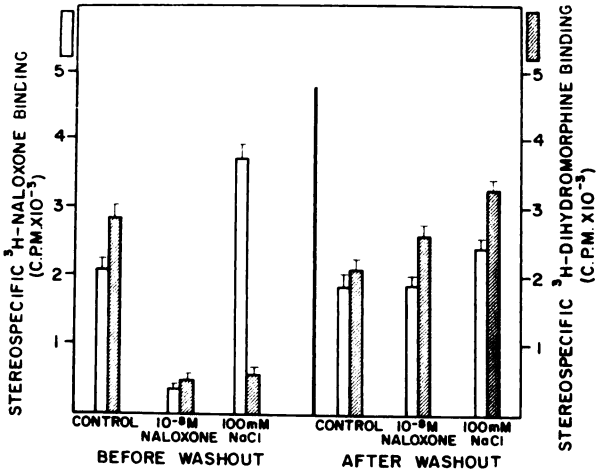

FIG. 3. *Reversibility of sodium chloride effect on [^{3}H]naloxone and [^{3}H]dihydromorphine stereospecific binding*

Standard rat brain homogenate (unwashed) was divided into four equal volumes and assayed for stereospecific [^{3}H]naloxone (2 nM) and [^{3}H]dihydromorphine (1 nM) binding in the presence of levallorphan and dextrallorphan (100 nM) after treatment as follows. Control, naloxone or sodium for 5 min at 25°. Treated homogenates: centrifuged at 40,000 × *g* for 5 min, after which the supernatant fluids were discarded and the pellets were reconstituted with cold 0.05 M Tris-HCl buffer (pH 7.4 at 37°) Once-washed homogenates: assayed identically at the same time; a typical experiment, which was replicated three times, is shown. An experiment that involved three additional centrifugations gave very similar results. Bars denote standard errors of the means.

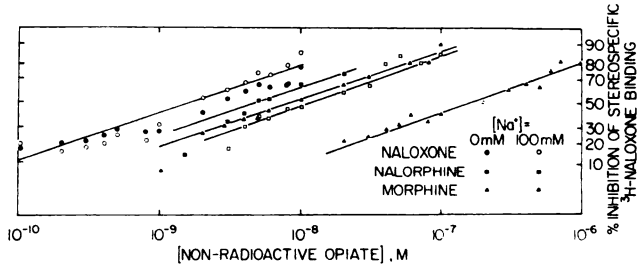

FIG. 4. *Log probit analysis of inhibition of stereospecific [^{3}H]naloxone binding in the absence and presence of sodium ion by nonradioactive naloxone, nalorphine, and morphine*

[^{3}H]Naloxone (1.5 nM) was incubated with standard aliquots of washed rat brain homogenate and 15 concentrations of each nonradioactive drug in triplicate for 30 min at 25° in the absence and presence of 100 mM NaCl. Control incubations, which contained nonradioactive levallorphan (100 nM) or dextrallorphan (100 nM), were included at the beginning and end of every experiment. Control stereospecific binding values were 1479 ± 121 and 3140 ± 300 cpm in the absence and presence of sodium chloride, respectively.

potency in the presence of sodium, while the potencies of their analogous antagonists are reduced less than 2-fold or not at all (Table 2). Thus, in the presence of Na^+, the relative affinities of agonists and antagonists for the receptor *in vitro* correspond closely with their relative potencies *in vivo*.

Influence of sodium on saturation of opiate receptor sites. A double-reciprocal analysis of the saturation of [^{3}H]naloxone binding is

TABLE 2

Effects of sodium ion 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 mM NaCl for 22 nonradioactive opiates, employing 100 nM (+)- and (−)-3-hydroxy-*N*-allylmorphinan to assess specificity. Rat brain, with the cerebellum removed, was homogenized (Polytron PT-10, 3000 rpm) in 100 volumes of 0.05 M Tris buffer and centrifuged at 40,000 × *g* for 10 min. After the supernatant fluid (which contained no specific binding activity) had been discarded, the pellet was reconstituted in the original volume of Tris buffer. Seven to ten concentrations of each drug were incubated with 1.5 nM [^{3}H]naloxone in the presence and absence of 100 mM NaCl. The concentration of drug that produced 50% inhibition of control stereospecific binding (EC_{50}) was determined by log probit analysis. Control [^{3}H]naloxone binding values in the absence and presence of 100 mM NaCl (0.05 M Tris-HCl buffer, pH 7.4, at 37°) were 1163 ± 104 and 2806 ± 198 cpm, respectively, at 44% counting efficiency.

Nonradioactive opiate	EC_{50} of stereospecific [^{3}H]naloxone binding		EC_{50} ratio, +NaCl/−NaCl	Pharmacological properties[a]	
	No NaCl	100 mM NaCl		Agonist	Antagonist
	nM	*nM*			
GPA 2163	100	20	0.2		1[b,c]
Naloxone	1.5	1.5	1.0		3[b-d]
Naltrexone	0.5	0.5	1.0		3[b-d]
Diprenorphine	0.5	0.5	1.0	3[c]	3[b,d]
N-Allylnormetazocine	2.0	3.0	1.5		3[b,d]
Cyclazocine	0.9	1.5	1.7	1[e], 3[f,g]	3[b,d]
Levallorphan	1.0	2.0	2.0	2[g]	3[b,d]
Nalorphine	1.5	4.0	2.7	1[e], 2[f,g]	2[b,d]
Pentazocine	15	50	3.3	1[e], 2[f,g]	1[b,d]
5-Propyl-5-normetazocine	12	50	4.2	2[e]	3[d], 1[b]
Metazocine	10	60	6.0	2[e,g]	1[b]
Etazocine	14	100	7.1	2[e,g]	1[b]
Etorphine	0.5	6.0	12	3[e,g]	
Phenazocine	0.6	8.0	13	3[e,g]	
Meperidine	3000	50,000	17	1[c,e,g]	
Levorphanol	1.0	15	15	3[c,e,g]	
Methadone	7.0	200	28	2[c,e,g]	
Oxymorphone	1.0	30	30	3[c,e,g]	
Morphine	3.0	110	37	2[c,e,g]	
Dihydromorphine	3.0	140	47	2[c,e,g]	
Normorphine	15	700	47	2[c,e,g]	
(±)-Propoxyphene	200	12,000	60	1[c,e,g]	

[a] The pharmacological properties of each opiate are designated as follows: 1, weak; 2, intermediate; 3, strong.

[b] Monkey abstinence precipitation (10, 18).

[c] Guinea pig intestine (19).

[d] Pierson-Harris tail flick (11, 20).

[e] Eddy-Leimbach mouse hot plate (21).

[f] Nilsen tail shock (22, 23).

[g] Human analgesia (24).

shown in Fig. 5. Between 0.14 and 2.1 nM [^{3}H]naloxone, sodium chloride (100 mM) produces a 2-fold increase in the maximal number of binding sites, with no apparent effect on affinity for naloxone. Thus 1 g of rat brain (without cerebellum) can bind 8 and 16 pmoles of naloxone in the absence and presence, respectively of 100 mM NaCl. Under both conditions the apparent K_d of naloxone binding is 1.4 nM. In earlier experiments, carried out before [^{3}H]naloxone of very high specific actitivy was available, we reported that receptor binding of naloxone had an apparent K_d of 20 nM and was relatively unaffected by high concentrations of Na^+ (12, 13). A recent report from this laboratory (32), in which a very wide range of naloxone concentrations was examined, revealed the presence of a low-affinity site unaffected by Na^+ and a high-affinity site enhanced by sodium ion.

Double-reciprocal analysis of [^{3}H]dihydromorphine binding describes a straight line (Fig. 6). The addition of 100 mM sodium chloride produces a 50% decrease in the number of binding sites, from 34 to 14 pmoles/g of rat brain without cerebellum, with no change in the apparent K_d of 20 nM. A high-affinity site with an apparent K_d of 2–3 nM can be observed in the absence of Na^+ (16, 32). Thus, at saturating concentrations, [^{3}H]dihydromorphine (assayed in the absence of Na^+) and [^{3}H]naloxone (in the presence and absence of Na^+) bind to a similar number of sites, 34 pmoles/g. This value is also in good agreement with the one reported by Simon *et al.* (33) for the potent agonist etorphine. These findings suggest that binding sites for opiate agonists and antagonists are identical and interconvertible. The parallel plots obtained for opiate agonists and antagonists in log probit analyses of their inhibition of [^{3}H]naloxone in the absence and presence of Na^+ (Fig. 4) also suggest that agonists and antagonists compete for the same population of binding sites. However, some deviations from strictly competitive inhibition are suggested in

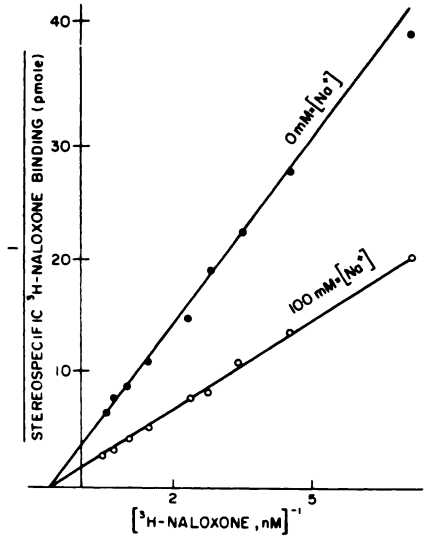

FIG. 5. *Effect of sodium chloride on saturation of stereospecific [^{3}H]naloxone binding*

After preparation of once-washed standard rat brain homogenate, aliquots were incubated with and without 100 mM NaCl with nine concentrations of [^{3}H]naloxone (0.14–2.1 nM), each in the presence of levallorphan (100 nM) and dextrallorphan (100 nM). The reciprocal of [^{3}H]naloxone concentration (abscissa) is plotted against the reciprocal of stereospecific [^{3}H]naloxone binding (ordinate).

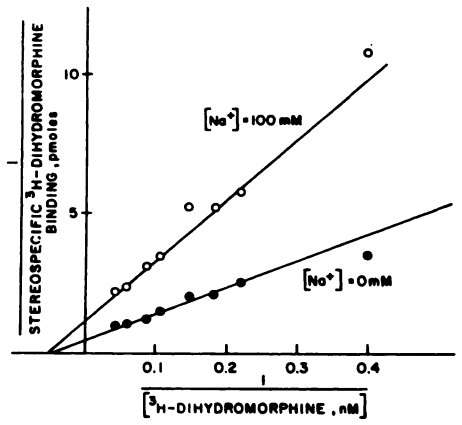

FIG. 6. *Effect of sodium chloride on saturation of stereospecific [^{3}H]dihydromorphine binding*

After preparation of once-washed standard rat brain homogenate, aliquots were incubated with and without 100 mM NaCl with eight concentrations of [^{3}H]dihydromorphine (55 Ci/mmole) between 2.4 and 23.8 nM. Each incubation was carried out in the presence of levallorphan (100 nM) and dextrallorphan (100 nM). The reciprocal of the [^{3}H]dihydromorphine concentration (abscissa) is plotted against the reciprocal of the stereospecific [^{3}H]dihydromorphine binding (ordinate).

Scatchard plots of levorphanol and etorphine inhibition of [^{3}H]naloxone binding at 100 mM NaCl (Fig. 7), which may be indicative of cooperative effects.

Effect of Na^+ on dissociation of [^{3}H]naloxone and [^{3}H]dihydromorphine binding from brain homogenate. The dissociation of [^{3}H]naloxone from rat brain homogenate examined in the presence of 10 μM nonradioactive naloxone describes a straight line when plotted semilogarithmically (Fig. 8). This result is consistent with a first-order dissociation function, as described previously (17). The rate of dissociation is the same in the presence or absence of Na^+ (Fig. 8). The half-life of dissociation, 8 min at 4°, is slower than that reported previously (17), since, at the low naloxone concentration employed here, binding at the high-affinity site predominates. The dissociation of [^{3}H]dihydromorphine, which appears to involve more than one component when plotted semilogarithmically, may be related to the crudeness of tissue preparation. Sodium ion markedly accelerates the initial rate of dissociation of [^{3}H]dihydromorphine. The half-life for dissociation of the first component of [^{3}H]dihydromorphine in the absence of Na^+ is about 8 min, while in the presence of Na^+ it is about 2.7 min.

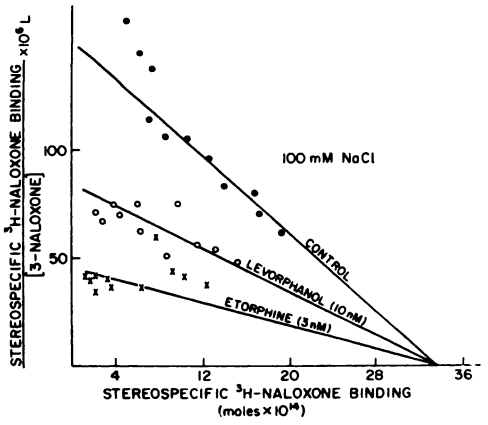

FIG. 7. *Analysis of inhibition of [^{3}H]naloxone binding by the agonists levorphanol and etorphine*

The standard rat brain homogenate, to which NaCl (100 mM, final concentration) was added, was incubated with 11 concentrations of [^{3}H]naloxone (0.3–3.0 nM) in the presence of levallorphan or dextrallorphan (100 nM). Etorphine (3 nM) and levorphanol (10 nM) were incubated with every concentration of naloxone. The amount of stereospecific [^{3}H]naloxone binding (moles) divided by the molar concentration of [^{3}H]naloxone in the medium (ordinate) is plotted against the amount of stereospecific [^{3}H]naloxone binding (abscissa).

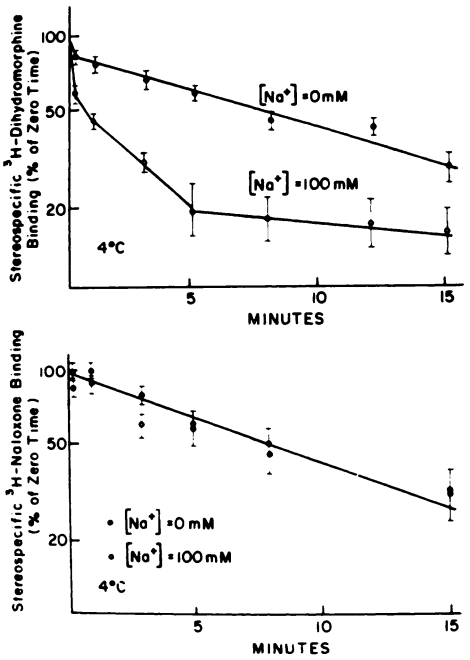

FIG. 8. *Dissociation of [^{3}H]dihydromorphine and [^{3}H]naloxone binding in the presence and absence of sodium ion at 4°*

Standard rat brain homogenate (washed once) was incubated with levallorphan or dextrallorphan at 100 nM and [^{3}H]naloxone (1.5 nM) or [^{3}H]dihydromorphine (1 nM) for 30 min at 25° in the absence of sodium ion. After cooling on ice, nonradioactive naloxone (lower figure) or dihydromorphine (upper figure) was rapidly added to a final concentration of 10 μM with or without NaCl (100 mM). Samples were maintained at 4° and filtered at various times after the addition of nonradioactive displacer. Values with the range indicated by bars are percentages of controls, to which no nonradioactive displacer was added.

Effect of temperature. We previously reported that [^{3}H]naloxone binding was temperature-dependent, with a 4-fold increase in specific binding from 4° to 25°, using 15-min incubations (17). However, in the presence of 100 mM NaCl less than 1.7-fold enhancement of [^{3}H]naloxone binding occurred when the temperature was raised from 5° to

35° (Fig. 9). In the absence of Na^+ [^{3}H]-dihydromorphine binding showed a temperature dependence resembling that of [^{3}H]-naloxone binding in the absence of Na^+, with 5-fold enhancement between 5° and 35°. Unlike the reduced temperature dependence shown by naloxone in the presence of Na^+, [^{3}H]dihydromorphine binding became much more temperature-dependent in the presence of Na^+, with 7-fold increments in binding between 5°, 15°, and 25°. Thus opiate receptor binding is highly temperature-dependent. Na^+ appears to make the binding of [^{3}H]naloxone energetically more favorable while simultaneously increasing the energy requirement for [^{3}H]dihydromorphine binding.

DISCUSSION

Sodium ion has been shown here to increase the binding of [^{3}H]opiate antagonists and to decrease the binding of [^{3}H]opiate agonists. Its ability to alter the degree to which nonradioactive opiates inhibit [^{3}H]-naloxone binding correlates impressively with the agonist-antagonist properties of these drugs. Certainly the designation of opiates as agonists, antagonists, or mixed agonist-antagonists is somewhat arbitrary, since this property is a continuum whose measurement is highly dependent on the particular test employed. Still, antagonists which possess little or no agonist activity inhibit naloxone binding to the same extent in the presence and absence of sodium, while increasing contamination of antagonists with agonist activity is correlated with an increasing sensitivity to the sodium-induced loss of inhibitory potency. "Pure" agonists without exception suffer a dramatic loss of inhibitory potency.

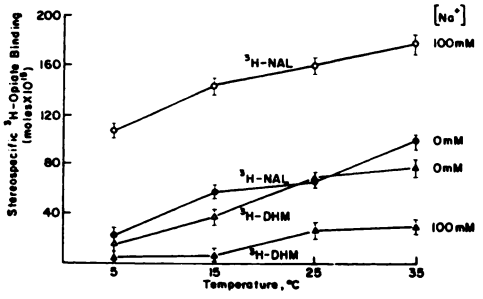

FIG. 9. *Temperature dependence of [^{3}H]naloxone (NAL) and [^{3}H]dihydromorphine (DHM) binding in the presence and absence of sodium ion*

Standard homogenates with or without 100 mM NaCl were incubated at the specified temperature (abscissa) for 5 min. After the addition of tritiated opiate (1 nM) the incubation was continued for 10 min at the same temperature. After cooling for 5 min in an ice bath (during which all samples attained a temperature of 4°) samples were filtered and washed as usual.

Interestingly, agonists differ quite a bit among themselves. Conceivably greater sensitivity to sodium is correlated with purer agonist qualities, whatever these may be. On the other end of the spectrum, the finding that GPA 2163 inhibits [^{3}H]naloxone binding with greater potency in the presence of Na^+ suggests that this experimental drug may possess properties of greater antagonist "purity" than naloxone, whatever these may be. Interestingly, Kosterlitz *et al.* (19) observed that slow dissociation from the guinea pig intestine correlates well with antagonist purity and that the dissociation rate of GPA 2163 is slower than that of naloxone.

The influence of sodium ion on opiate receptor binding is highly specific. Lithium ion, whose atomic radius and biological activity are similar to those of Na^+ is the only other ion which can mimic its actions. The other monovalent cations, potassium, rubidium, and cesium, cannot discriminate between agonists and antagonists. In addition, a variety of divalent cations and anions also fail to discriminate between binding of opiate agonists and antagonists. The highly specific nature of the sodium effect suggests that this ion interacts with sites on the membrane which can allosterically transform the opiate receptor. Presumably the binding of Na^+ induces a conformational change which renders the receptor site less likely to bind agonists and more likely to bind antagonists. One explanation of the finding that Na^+ causes a 3-fold acceleration of dihydromorphine dissociation without altering the rate of naloxone dissociation is that the ion transforms the receptor site into a conformation which promotes the dissociation of agonists.

A "disappearance" of dihydromorphine

binding sites with a concurrent "appearance" of naloxone binding sites occurs when Na^+ is added to the medium. Together with the marked temperature dependence of binding, this suggests that opiate receptors exist in an equilibrium between two distinct conformations. One, which is favored in the presence of Na^+, binds antagonists with greater affinity, while another conformation binds agonists selectively with greater affinity and is favored in the absence of Na^+.

General theoretical cooperative drug models have been proposed (33) in which the relative affinity for each of two conformations is reflected as intrinsic activity or effieacy. Karlin (35) has described a model in which the binding of agonists or antagonists produces a concerted transition between two conformational states. According to this model the physiological consequences of opiate agonist binding would result from the reduced ability of the membrane to bind sodium ion.

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