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The Interaction of Phenoxybenzamine with the Mouse Brain Opiate Receptor

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SUMMARY

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Phenoxybenzamine was found to displace stereospecifically bound [^{3}H]naloxone from mouse brain homogenates in a dose-related manner, with an IC_{50} of 4.6 μM. In the presence of 100 mM Na^+, the dose-response curve shifted to give an IC_{50} of 19 μM and an Na^+ response ratio of 4.3, which is similar to that of a mixed agonist-antagonist. Scatchard plots of the binding data showed that phenoxybenzamine reduced the number of receptor binding sites for naloxone but did not change the affinity of the unreacted receptors for naloxone. Levallorphan protected the receptor from phenoxybenzamine, but the inhibition of binding activity produced by phenoxybenzamine could not be reversed by repeated washing of the receptor preparation. It thus appears that phenoxybenzamine acts as an irreversible, non-equilibrium inhibitor with binding characteristics similar to those of the agonist-antagonist narcotics. Washed brain homogenates from mice treated *in vivo* with antinociceptive doses of phenoxybenzamine exhibited enhanced binding of [^{3}H]naloxone and [^{3}H]morphine, which may indicate the displacement of an endogenous material from the opiate receptors *in vivo* by phenoxybenzamine.

INTRODUCTION

Phenoxybenzamine has often been used in the study of norepinephrine involvement in morphine analgesia (e.g., refs. 1 and 2). It has been assumed in such studies that phenoxybenzamine acts in antinociception primarily as an *alpha* adrenergic blocking agent. Phenoxybenzamine itself has been reported to be antinociceptive (1, 3). We have reported that the antinociceptive action of phenoxybenzamine in the mouse writhing assay is antagonized by naloxone (4). Phenoxybenzamine has also been reported to inhibit contraction of the stimulated guinea pig ileum, which was blocked by naloxone (5). The ability of naloxone, a pure narcotic antagonist, to shift the analgesic dose-response curve of phenoxybenzamine to the right (4) suggests that phenoxybenzamine may act at the same site as morphine rather than at a parallel or serial site of *alpha* adrenergic receptors.

Cicero *et al.* (6) have reported that phenoxybenzamine inhibited the specific binding of naloxone to brain homogenates. The objective of this study was to investigate the possible interaction of phenoxybenzamine with the brain opiate receptor.

MATERIALS AND METHODS

Levorphanol, levallorphan, dextrorphan, and (−)-2-hydroxy-*N*-cyclopropylmethylmorphinan HCl (Ro 20-2230) were supplied

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by W. Scott, Hoffmann-La Roche; pentazocine, by Sterling Drug; and butorphanol, by William F. Minor, Bristol Laboratories.

Male Swiss-Webster mice (Simonsen Laboratories, Gilroy, Cal.) weighing 20–30 g were used for all experiments. Each animal was decapitated and the brain was rapidly removed. The cerebellum was removed, the remaining brain was homogenized, and stereospecific naloxone binding was determined according to Pert *et al.* (7). In most experiments, 3–12 brains were pooled and homogenized in ice-cold Tris–EDTA buffer (50 mM Tris and 5 mM EDTA, pH 7.4, at 37°; 1 g of brain tissue in 20 ml of buffer). [^{3}H]Naloxone (New England Nuclear; specific activity, 19.985 Ci/mmole), 1 nM, was used. A 15-min preliminary incubation with drugs and a 30-min incubation after naloxone were carried out in all but two experiments: the experiment on the effect of varying preliminary incubation times and the redetermination of the IC_{50} using a 2-hr incubation.

Triplicate tubes containing 100 nM dextrorphan (total binding) or 100 nM levorphanol (nonstereospecific binding) and 1 nM [^{3}H]naloxone were assayed. The difference between the average counts per minute in the total and nonspecific binding tubes was taken to be the total stereospecific binding in that preparation. Total and nonspecific binding was determined with each series. The percentage of stereospecific binding naloxone displaced by a test dose of drug was calculated as follows:

$$\text{Total tube dpm} - \text{nonspecific tube dpm} = \text{stereospecific naloxone binding}$$

$$\text{Total tube dpm} - \text{drug-unknown tube dpm} = \text{drug-occupied binding sites}$$

$$\frac{\text{Drug-occupied binding sites}}{\text{Stereospecific naloxone binding sites}} \times 100 = \%\ \text{stereospecific naloxone binding displaced by drug}$$

Stereospecific [^{3}H]naloxone binding is defined as that [^{3}H]naloxone binding which can be displaced by levorphanol but not by dextrorphan. In determination of the Na^+ response ratio, levallorphan was used in place of levorphanol to determine nonspecific binding. Stereospecific binding was 50–85% of total binding.

As a control to test whether phenoxybenzamine would inhibit nonspecific [^{3}H]-naloxone binding, the [^{3}H]naloxone binding to homogenates incubated with levorphanol and phenoxybenzamine was compared with binding to a homogenate incubated with levorphanol or phenoxybenzamine alone. In a typical experiment the average radioactivity in the levorphanol-phenoxybenzamine tube was 1840 cpm; for levorphanol alone, 1900 cpm; for phenoxybenzamine alone, 1800 cpm; and for dextrorphan alone, 3600 cpm. Binding for phenoxybenzamine alone (1800 cpm) was not significantly different from binding for phenoxybenzamine and dextrorphan (1830 cpm). From these results it appears that phenoxybenzamine inhibits the same binding that levorphanol does; i.e., the stereospecific binding sites.

RESULTS

Displacement of stereospecific naloxone binding by phenoxybenzamine. Phenoxybenzamine appeared to displace [^{3}H]naloxone from stereospecific binding sites in brain homogenates in a dose-related manner (Fig. 1). From the 15-min preliminary incubation and the 30-min incubation, the IC_{50} was 4.59 μM. From the dose-response curve determined for the 2-hr incubation (maximum displacement), an IC_{50} of 1.2 μM was obtained. In our assay, morphine had an IC_{50} of 16.1 nM (see Table 1). The ratio of the IC_{50} values of morphine and phenoxybenzamine in this binding assay was about 100, while in the mouse writhing test the ratio of the ED_{50} of morphine to that of phenoxybenzamine was approximately 30 (4).

Pert *et al.* (7) have shown that log-probit plots of concentration vs. stereospecifically bound naloxone displaced are parallel for a large number of opiate agonists and antagonists. The log-probit dose-response curve for phenoxybenzamine shown in Fig. 1 was parallel to those of opiates tested. The slope of the curve for phenoxybenzamine, 0.58, was not significantly different, using the criterion $p < 0.05$, from that for morphine, 0.59; butorphanol, 0.54; Ro 20-2230 (7, 8), 0.56; or levorphanol, 0.61 (data not shown).

In order to determine the nature of phe-

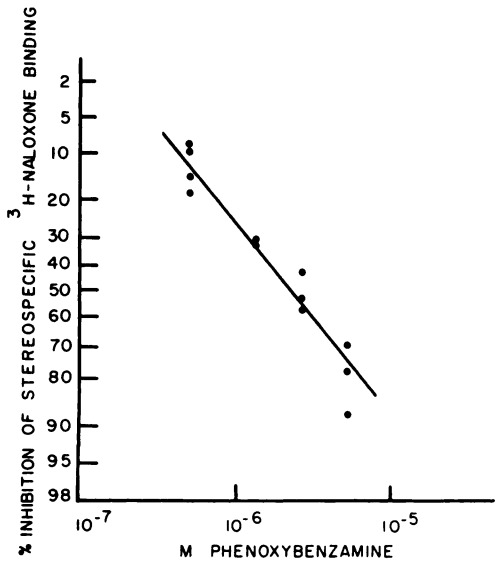

FIG. 1. *Dose-response relationship of phenoxybenzamine inhibition of stereospecific [^{3}H]naloxone binding*

A brain homogenate was incubated with various concentrations of phenoxybenzamine for 90 min at 25°. Then 1 nM [^{3}H]naloxone was added, and incubation was continued for 30 min. Aliquots (2 ml) of the homogenate were chilled, filtered on GF/B filters over vacuum, and washed two times with cold buffer. Filters were dispersed in Ready Solv liquid scintillation counting solution and counted for 10 min each on a Beckman LS-100C liquid scintillation counter. Concentrations are plotted as logarithms, and percentage inhibition of stereospecific [^{3}H]naloxone binding, as probits. The dose-response curve was fitted by least-squares linear regression analysis.

noxybenzamine interaction with the brain morphine receptor, the influence of phenoxybenzamine on saturation binding curves for stereospecific binding of naloxone was determined (Fig. 2A). A Scatchard plot of the same data is shown in Fig. 2B. The average value for K_d for naloxone binding in the absence of phenoxybenzamine from Scatchard plot analysis was 1.45 ± 0.61 nM (N = 7). In the presence of phenoxybenzamine (13.5 or 5.5 μM) the number of stereospecific sites available for naloxone binding (n) was reduced to 25% and 50% of control, respectively. However, the average value of K_d for naloxone binding in the presence of phenoxybenzamine, 1.99 ± 1.029 nM (N = 4), was not significantly different ($p < 0.05$) from that found for naloxone alone.

Displacement of stereospecific [^{3}H]naloxone binding of other alpha adrenergic blockers. The *alpha* adrenergic blocker tolazoline, in concentrations from 0.1 to 50

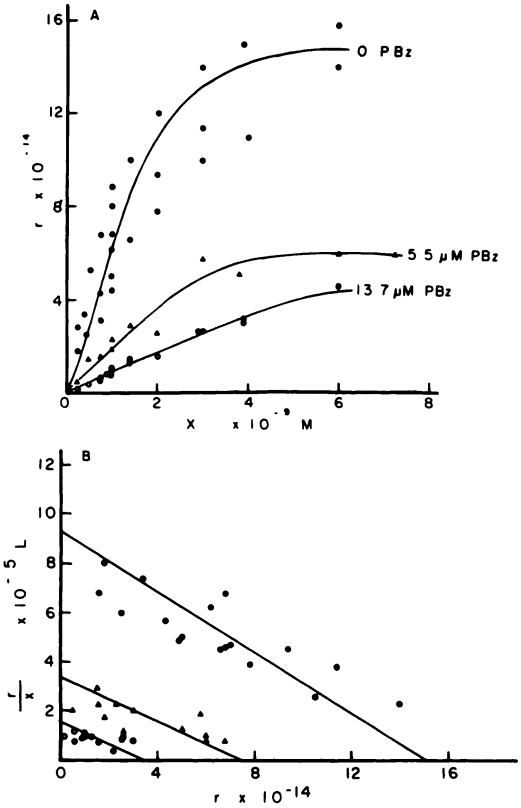

FIG. 2. *Binding of [^{3}H]naloxone in the presence and absence of phenoxybenzamine*

A. Saturation binding curves. Mouse brain homogenate (washed) was incubated with 0.25, 0.50, 0.75, 1.0, 1.5, 2, 3, 4, or 6 nM [^{3}H]naloxone in the absence (●) and presence of 5.5 (▲) or 13.7 (○) μM phenoxybenzamine (PBz), and stereospecific [^{3}H]naloxone binding was determined as described under MATERIALS AND METHODS. Moles of naloxone bound per 20 mg of tissue are plotted against the concentration of [^{3}H]-naloxone added to the homogenate. These data are from three typical experiments performed on successive days.

B. Scatchard plot of the same data. The ratio of moles of [^{3}H]naloxone bound to the concentration of [^{3}H]naloxone added is plotted against moles of [^{3}H]naloxone bound. Lines were fitted by least-squares linear regression analysis. For these data, in the absence of phenoxybenzamine (●), for [^{3}H]naloxone alone, $n = 15.0 \times 10^{-14}$ mole/20 mg of tissue, K = 2.1 nM; in the presence of 5.5 μM phenoxybenzamine (▲), $n = 7.5 \times 10^{-14}$ mole/20 mg of tissue, K = 2.2 nM; in the presence of 13.7 μM phenoxybenzamine (○), $n = 2.8 \times 10^{-14}$ mole/20 mg of tissue, K = 2.4 nM.

μM, was tested in the presence and absence of 100 mM NaCl but did not displace [^{3}H]-naloxone binding in the brain homogenates at the concentrations tested (Table 1). Phentolamine (1 nM–1 mM) was also added to the brain homogenates, and over the range from 1 nM to 10 μM it did not replace [^{3}H]naloxone binding. However, 0.5 mM phentolamine displaced 50% of the stereospecific naloxone binding (Table 1).

Na^+ response ratio. The effect of Na^+ on the phenoxybenzamine dose-response curve was determined. In the presence of 100 mM Na^+, the dose-response curve shifted to the right, resulting in an IC_{50} of 19 μM (see Fig. 3). The ratios of the IC_{50} in the presence of 100 mM Na^+ to the IC_{50} in the absence of Na^+ determined for phenoxybenzamine, several narcotic agonists, and partial agonists in this assay are shown in Table 1. These ratios indicate that phenoxybenzamine has effects on binding similar to those of the mixed agonist-antagonists such as pentazocine, Ro 20-2230, and butorphanol. The narcotic antagonist analgesic Ro 20-2230 (a 2-hydroxymorphinan) is approximately equal to pentazocine as an analgesic in the mouse writhing test and 3 times more potent than pentazocine as a morphine antagonist in the mouse tail flick test (8, 9). The narcotic antagonist analgesic butorphanol (a 3,14-dihydroxymorphinan) is 30 times more potent than pentazocine as an analgesic in the mouse writhing test and 30 times more potent than pentazocine as a narcotic antagonist (10).

Time course of phenoxybenzamine interaction with brain opiate receptor. Various concentrations of phenoxybenzamine were added to the brain homogenate before and after addition of naloxone, and the influence of incubation times was investigated (Fig. 4). The concentration of phenoxybenzamine and the total time of incubation of phenoxybenzamine with the homogenate determined the percentage of stereospecifically bound naloxone displaced. The order of addition of the labeled naloxone did not alter the displacement of apparent stereospecifically bound naloxone. In Fig. 4, this is shown by the open circles (preliminary incubation with labeled naloxone) coinciding within experimental error with the solid circles (naloxone added after incubation with phenoxybenzamine).

The percentage of stereospecifically bound naloxone displaced by a given concentration of phenoxybenzamine increased up to 2 hr of incubation and remained constant or decreased slightly after 3 hr or more (2.5, 3, and 4 hr) (Fig. 4). That this plateau was not due to degradation of the

TABLE 1

IC_{50} values and Na^+ response ratios for some opiates and alpha adrenergic blockers

Dose-response curves for the inhibition of stereospecific [^{3}H]naloxone binding were determined in the presence and absence of 100 mM NaCl for nine compounds, using 100 nM levallorphan and dextrorphan to assess specificity. A brain homogenate was incubated with six to nine concentrations of each drug and 1.0 nM [^{3}H]naloxone in the presence and absence of 100 mM NaCl. Percentage inhibition of stereospecific [^{3}H]naloxone binding was determined as described in MATERIALS AND METHODS. The IC_{50} (50% inhibition of stereospecific naloxone binding) for each drug was determined by least-squares linear regression analysis of log-probit transformation of concentrations and percentage inhibition. Numbers in parentheses indicate the number of replicate experiments performed.

Drug	IC_{50}		Ratio
	$-Na^+$	$+Na^+$	
	n M	*n M*	
Butorphanol	6.02 (4)	10.69 (6)	1.78
Phenoxybenzamine	4,590 (9)	19,600 (5)	4.27
Ro 20-2230	31.8 (5)	182.02 (5)	5.72
Pentazocine	32.9 (5)	227.4 (5)	6.91
Levorphanol	2.32 (6)	31.59 (6)	13.6
Morphine	16.09 (9)	237.0 (5)	14.7
Dextrorphan	12,000 (3)		
Phentolamine	500,000 (3)		
Tolazoline	0 (3)		

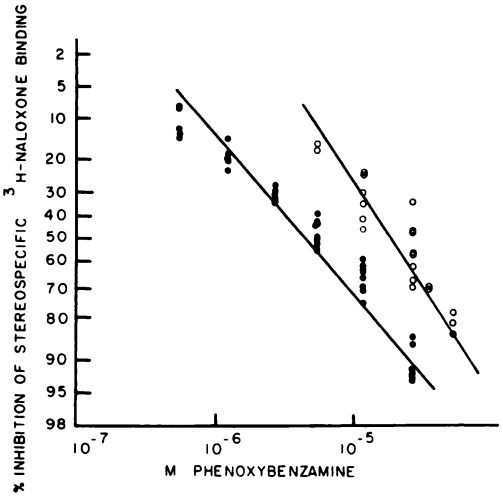

FIG. 3. *Effect of Na^+ on dose-response curve for phenoxybenzamine*

Inhibition of stereospecific [^{3}H]naloxone binding was determined in the presence (○) and absence (●) of 100 mM NaCl for eight concentrations of phenoxybenzamine, using 100 nM levallorphan and dextrorphan to assess specificity. Mouse brains, with cerebella removed, were homogenized in 50 mM Tris–5 mM EDTA buffer, pH 7.4, at 37°, and centrifuged at 18,000 × *g* for 10 min. The supernatant was discarded, and 3 ml of the resuspended pellet (20 mg of tissue per milliliter of buffer) were incubated at 25° with each drug concentration (15-min preliminary incubation) and 1.0 nM [^{3}H]naloxone (30-min incubation) in the presence and absence of 100 mM NaCl. Percentage inhibition was determined as described in MATERIALS AND METHODS. The IC_{50} (50% inhibition of stereospecific [^{3}H]naloxone binding) was determined by least-squares linear regression analysis of the log-probit transformation of concentrations and percentage binding.

receptor preparation was shown by the constant value throughout this time for the total stereospecific naloxone binding sites determined using the dextrorphan and levallorphan saturation tubes (data not shown).

Dissociation of phenoxybenzamine binding. To determine the ease of dissociation of phenoxybenzamine binding, the effect of washing after incubation with the drug was investigated. After washing, the stereospecific naloxone binding displaced by the drug was compared with stereospecific binding of naloxone in a portion of the homogenate that had been washed the same number of times but had not been exposed to preliminary incubation with the drug (Fig. 5). Levallorphan, a reversibly bound opiate, was used as a control to measure the effectiveness of washing.

Although less than 20% of the levallorphan appeared to remain to displace naloxone binding after five washes, the incubation with phenoxybenzamine appeared to render 80% of the receptors inaccessible to naloxone even after five washings. Thus the interaction of phenoxybenzamine with the opiate receptor is apparently irreversible.

Protection of stereospecific binding sites against phenoxybenzamine by levallorphan. The addition of sufficiently high concentrations of levallorphan during the initial incubation prevented the inhibition of stereospecific binding caused by phenoxybenzamine as measured after five washes to remove unbound or reversibly bound drug (Table 2). The effect was proportional to the concentration of levallorphan added in the initial incubation (Table 2, experiment A).

Alternatively, the concentration of phenoxybenzamine was varied and the concentration of levallorphan was held constant at 100 μM in the initial incubation. The recovery of stereospecific naloxone binding was increased in each case by the addition of

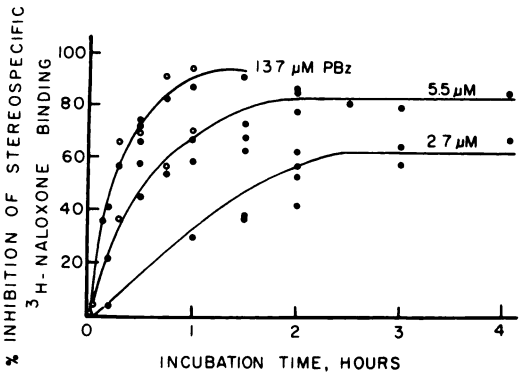

FIG. 4. *Effect of incubation time on inhibition of [^{3}H]naloxone binding by phenoxybenzamine*

The standard mouse brain homogenate (washed) was incubated for 3 min–4 hr with 13.7, 5.5, 2.75, 1.37, or 0.55 μM phenoxybenzamine (PBz) and 1 nM [^{3}H]-naloxone at 25°. Percentage inhibition of stereospecific [^{3}H]naloxone binding was determined as described in MATERIALS AND METHODS. ●, incubation with unlabeled drug first and then with labeled naloxone; ○, incubation with labeled naloxone first and then drug.

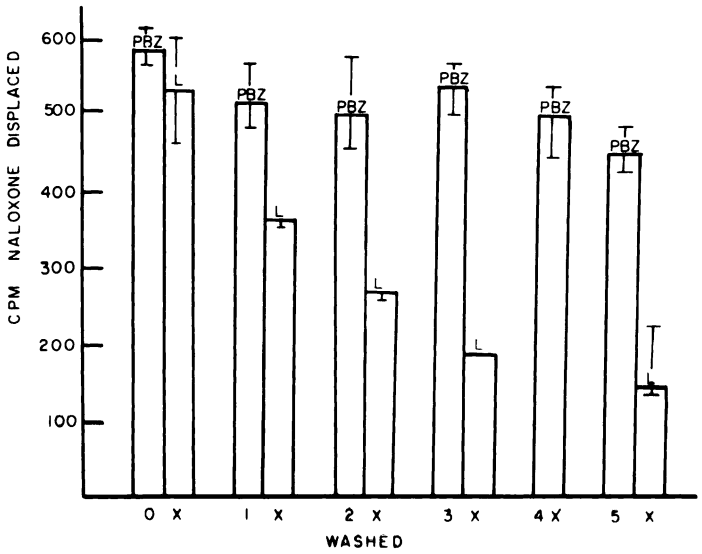

FIG. 5. *Effect of washing on inhibition of [^{3}H]naloxone binding by phenoxybenzamine*

Mouse brains, with cerebella removed, were homogenized with 50 mM Tris–5 mM EDTA buffer, pH 7.4, at 37° and centrifuged at 18,000 × *g* for 10 min. The supernatant was discarded, and the pellet was resuspended at 20 mg of tissue per milliliter of buffer. The homogenate was incubated with 55 μM phenoxybenzamine (PBz) or 0.1 μM levallorphan (L) for 30 min, then centrifuged at 5° and resuspended in ice-cold fresh buffer five times. After each wash aliquots were removed and stereospecific [^{3}H]naloxone binding was determined as described in MATERIALS AND METHODS. Vertical bars show standard deviations. The experiment was replicated three times.

levallorphan in the initial incubation (Table 2, experiment B).

Effect of administration of phenoxybenzamine in vivo on brain opiate receptor activity. The total stereospecifically bound naloxone was increased in brain homogenates obtained from the phenoxybenzamine-treated animals, compared with controls subjected to the same procedures (Table 3A). This apparent enhancement of brain opiate receptor activity was greater after three washes of the homogenate. Increasing the dose of phenoxybenzamine administered to the mice to 500 mg/kg did not increase the magnitude of the apparent enhancement of receptor activity, nor did it cause inactivation of the receptor. The enhancement of naloxone binding was observed 0.5 hr after treatment and was not increased by waiting until 2.5 hr. A parallel experiment was performed in which binding of an agonist, [^{3}H]morphine, was also determined (Table 3B). At 0.5 hr (a time at which the mice showed no antinociceptive effect) after subcutaneous injection of phenoxybenzamine, the brain homogenate binding of [^{3}H]morphine showed no change or a decrease in binding compared with binding to brain homogenates of controls injected with vehicle. At 2.5 hr after drug injection [the time of peak phenoxybenzamine antinociceptive action (4)], the brain homogenate from treated animals bound on the average 50% more [^{3}H]morphine than the brain homogenate from control mice.

DISCUSSION

The inhibitory effects of phenoxybenzamine on stereospecific naloxone binding *in vitro* are consistent with naloxone antagonism of phenoxybenzamine antinociception, as previously reported (4), which would now appear to be due to interaction of naloxone and phenoxybenzamine at the opiate receptor. Furthermore, the failure of other *alpha* adrenergic blocking agents to inhibit naloxone binding is consistent with the failure of naloxone to block the antinociceptive actions of *alpha* adrenergic agonists and antagonists (2, 4). Our value for the IC_{50} of phenoxybenzamine in inhibiting stereospecific naloxone binding, 1.2 μM, is

TABLE 2

Protection of opiate receptor by levallorphan from irreversible inhibition by phenoxybenzamine

Brain homogenate was incubated for 0.5 hr with 13.7, 27.5, or 55 μM phenoxybenzamine and/or 0.1, 1.0, 10, or 100 μM levallorphan. The homogenate was then centrifuged at 18,000 × *g* for 10 min and resuspended in Tris buffer. This procedure was repeated five times to remove unbound and reversibly bound drugs. Then 1 nM [^{3}H]naloxone added to the final suspension, and stereospecific [^{3}H]naloxone binding was determined as described in MATERIALS AND METHODS.

Expt.	Phenoxybenzamine	Levallorphan	Stereospecific	[^{3}H]naloxone binding
	μM	*μM*	*cpm*	*% control*
A	55	0	55	8
	55	0.1	145	22
	55	1.0	246	37
	55	10	252	38
	55	100	382	57
	0	10	670	100
	0	100	671	100
B	55	0	55	8
	55	100	382	57
	27.5	0	167	27
	27.5	100	345	56
	13.7	0	393	64
	13.7	100	625	101
	0	100	617	100

in reasonable agreement with the value of 0.92 μM reported by Cicero *et al.* (6).

The parallelism of the dose-response curves for phenoxybenzamine and for opiates in displacing stereospecific naloxone binding is also consistent with, but not proof of, binding of phenoxybenzamine to the opiate receptor. This is further reinforced by the Scatchard analysis (Fig. 2A and B), which indicates that phenoxybenzamine reduces the number of available binding sites but does not alter the affinity of the unreacted receptors for naloxone. These effects on the opiate receptor closely resemble the effects of phenoxybenzamine on the *alpha* adrenergic receptor *in vitro* reported by Williams and Lefkowitz (11), who concluded that phenoxybenzmine acts in a non-equilibrium, irreversible manner with the receptor.

Further evidence that phenoxybenzamine interacts with the opiate receptor is the finding that 1–100 μM levellorphan protected the receptor from inactivation by phenoxybenzamine. Since washing of the brain preparation after incubation with phenoxybenzamine did not reactivate the opiate receptor, it would appear that the interaction with phenoxybenzamine is essentially irreversible. This is consistent with the noncompetitive or non-equilibrium binding revealed by the kinetic analysis in Fig. 2A and B.

Pert, Pasternak, and Snyder (12, 13) have shown that opiate agonists and antagonists can be differentiated in the brain opiate receptor assay *in vitro* by the shift in binding produced by addition of 100 mM NaCl to the assay medium. They suggested that Na^+ alters the equilibrium between receptor configurations favorable for binding of agonists and antagonists. When the effects of phenoxybenzamine inhibition of [^{3}H]naloxone binding were examined in the presence and absence of 100 mM Na^+ (Table 1), a ratio of 4.27 was obtained, which places phenoxybenzamine with the known mixed agonist-antagonists such as pentazocine. Pert and Synder (14) also reported that antagonists were 10–100 times more potent than agonists in enhancement of receptor binding after administration *in vivo*. Since in the mouse writhing assay morphine is 30 times more potent an analgesic than phenoxybenzamine but is less than 10 times as potent in enhancing brain opiate receptor activity, this also suggests that phenoxybenzamine acts as an antagonist as well as an agonist in interaction with the opiate receptor. This has now been confirmed in animal tests of analgesia and antagonism (15, 16). Using the standard tail flick assay with a control latency of 2 sec and a cutoff time of 12.5 sec, phenoxybenzamine did not significantly prolong tail flick latency. However, when the modified tail flick assay developed by Gray *et al.* (17) for the measurement of pentazocine and other antagonist analgesics was employed, phenoxybenzamine was found to prolong the tail flick latencies significantly ($P \leqq 0.01$). Also, phenoxybenzamine had approximately 1/20 the potency of morphine in the tail flick assay (15). In screening tests for antagonism, phenoxybenzamine was found to antagonize the oxymorphine-induced Straub tail reaction in low doses, to antagonize

TABLE 3

Enhancement of brain morphine receptor binding activity after drug treatment in vivo

Mice were given 100 mg/kg of phenoxybenzamine or similarly diluted vehicle subcutaneously and were killed 0.5 or 2.5 hr after treatment. This dose of phenoxybenzamine causes a 95% or greater reduction of stretching in the mouse stretching reflex analgesia test (4). The stereospecific binding of [^{3}H]naloxone was determined in pooled brain homogenates of three treated and three control mice after a single wash and after three washes to remove any drug still present in the brain. Stereospecific [^{3}H]naloxone binding was determined as described in MATERIALS AND METHODS, in triplicate for each experiment. Counts per minute per 20 mg of original brain tissue reported below are averages of the triplicates of each day's experiment. Determination of stereospecific [^{3}H]morphine binding was carried out as described in MATERIALS AND METHODS, with the substitution of 13 nM [^{3}H]morphine for 1 nM [^{3}H]naloxone and of 10 μM unlabeled morphine for 0.1 μM levorphanol.

Time after treatment	Expt.	Washes[a]	Phenoxybenzamine	Control	Change	p
hr			*cpm + SD*		%	
A. Stereospecific naloxone binding						
0.5	1	1	1085 ± 33	901 ± 42	+20.4	≤0.02
	2	1	1380 ± 50	1325 ± 107	+4.7	NS[b]
	3	1	1278 ± 215	1191 ± 33	+7.3	NS
					Av. 10.8 ± 8	
	4	3	942 ± 22	801 ± 28	+17.6	≤0.01
	5	3	1063 ± 81	703 ± 19	+51.4	≤0.01
	6	3	1024 ± 65	711 ± 130	+43.2	≤0.05
					Av. 37.4 ± 17	
2.5	7	1	742 ± 44	693 ± 55	+7.0	NS
	8	1	875 ± 59	860 ± 89	0	NS
					Av. 4.9 ± 4	
	9	3	781 ± 54	657 ± 46	+15.5	≤0.05
	10	3	978 ± 70	669 ± 60	+46.5	≤0.02
	11	3	1099 ± 44	900 ± 46	+22.2	≤0.02
					Av. 28.1 ± 16	
B. stereospecific morphine binding						
0.5	12	3	1519 ± 168	1541 ± 404	−1.4	NS
	13	3	841 ± 209	1009 ± 154	−16.6	NS
	14	3	1099 ± 94	1112 ± 130	−1.2	NS
					Av. −6.4 ± 8	
2.5	15	3	2036 ± 330	1151 ± 129	+76.8	≤0.05
	16	3	1290 ± 207	905 ± 240	+42.5	≤0.1
	17	3	1222 ± 245	1012 ± 283	+20.6	≤0.1
					Av. 46.8 ± 23	

[a] Centrifugation and resuspension in ice-cold 50 mM Tris–5 mM EDTA buffer, pH 7.4, at 37°.

[b] Not significant at $p \leq 0.1$.

morphine analgesia in the mouse writhing assay, and to induce abstinence syndrome in nonwithdrawn morphine-dependent mice (16).

Simon *et al.* (18) showed that the Na^+-induced change in conformation can be correlated with a change in the accessibility of a critical sulfhydryl group to agents such as *N*-ethylmalemide, presumably because of protection of the sulfhydryl group in the Na^+-induced configuration. The increase in the phenoxybenzamine concentration needed to displace naloxone in the presence of Na^+ might be due to the Na^+-induced conformational protection of the sulfhydryl group postulated by Simon *et al.* (18). This may be significant, since it is known that phenoxybenzamine or its active ethylenei-

monium ion preferentially reacts with sulfhydryl groups to form a covalent complex (19, 20), and since in its reaction as an *alpha* adrenergic blocker phenoxybenzamine has been postulated to form first a reversible weak bond with the adrenergic receptor and then to form slowly a covalent bond via an essential sulfhydryl group on that receptor (11).

Although the interaction of phenoxybenzamine with the opiate receptor has been documented by these experiments *in vitro*, it is important to assess the pharmacological significance of such interactions in the antinociceptive action of phenoxybenzamine. Table 3 shows that after phenoxybenzamine injection (100 mg/kg subcutaneously) the opiate receptor binding activity for [^{3}H]morphine and [^{3}H]naloxone was enhanced rather than diminished. It may be significant that the degree of enhancement of [^{3}H]morphine binding was greater when examined at 2.5 hr than at 0.5 hr after phenoxybenzamine administration, since this change with time parallels the antinociceptive activity of phenoxybenzamine, which was barely detectable at 0.5 hr but peaked at 2.5 hr after phenoxybenzamine injection (4). The mechanism of the unexpected enhancement caused by phenoxybenzamine *in vivo* is not explained by the properties revealed *in vitro* but may be related to the enhancement described by Pert and Snyder (14) for opiates.

Therefore the results of tests both *in vitro* and *in vivo* provide evidence that phenoxybenzamine can interact with the opiate receptor and suggest that the antinociceptive action of this compound and its effect on morphine analgesia (1, 4) could be due to its interaction at the brain opiate receptor rather than at brain *alpha* adrenergic receptors.

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