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Sites of Action of Phencyclidine

III. Interactions with Muscarinic Receptors

ROBERT S. ARONSTAM,[1] MOHYEE E. ELDEFRAWI, AMIRA T. ELDEFRAWI, EDSON X. ALBUQUERQUE, KAM F. JIM* AND DAVID J. TRIGGLE*

*Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, Maryland 21201, and *Department of Biochemical Pharmacology, School of Pharmacy, State University of New York at Buffalo, Amherst Campus, Buffalo, New York 14260*

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SUMMARY

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The effects of phencyclidine (PCP) and phencyclidine methiodide (PCP-MeI) on contractions of longitudinal muscle of guinea pig ileum and specific binding of [^{3}H](DL)3-quinuclidinyl benzilate ([^{3}H]QNB) to muscarinic receptors in these muscles and rat brain were studied. PCP inhibited competitively muscarinic agonist-induced contractions of the longitudinal muscle of guinea pig ileum at concentrations below 10 μM (K_i = 0.45 μM). However, at 50 μM noncompetitive blockade of contraction was seen. In contrast, PCP-MeI inhibited ileum contractions competitively at concentrations of up to 100 μM (K_i = 0.2 μM). Both drugs inhibited binding of [^{3}H]QNB to muscarinic acetylcholine (ACh) receptors of rat cerebral cortex and brain stem and to ileal longitudinal muscle (apparent K_i's of from 0.8 to 3.7 μM). There was no evidence of noncompetitive receptor binding inhibition at PCP concentrations which produced noncompetitive inhibition of smooth muscle contraction. There were no indications of multiple receptor populations, cooperative interactions, or receptor isomerization with either drug. PCP-MeI was slightly more potent in its competitive muscarinic actions than PCP. Although the dissociation constant (K_d) of PCP from muscarinic receptors in brain cortex was significantly lower than in brain stem, the K_d values for PCP-MeI were the same. Treatment with *N*-ethylmaleimide did not affect the inhibition of [^{3}H]QNB binding to muscarinic receptors by PCP. Based on several characteristics of the inhibition by PCP and PCP-MeI of [^{3}H]QNB binding to rat brain muscarinic receptors and their inhibition of the contractions of longitudinal muscle of guinea pig ileum, PCP and PCP-MeI are considered antagonists of muscarinic receptors in rat brain.

INTRODUCTION

There is ample evidence that phencyclidine (PCP)[2] and its derivatives have cholinergic actions (1–8). The effect of PCP on the ionic channel of the acetylcholine (ACh) receptor is evident from biophysical data on amphibian and mammalian skeletal muscles and biochemical data on fish electric organs (1, 2). Although PCP does not inhibit the reaction of ACh or α-bungarotoxin with the nicotinic receptors in innervated and denervated muscles, or *Torpedo* electric organ (1), it alters the properties of the ionic channel of the ACh receptor and inhibits its binding of perhydrohistrionicotoxin. PCP and its methiodide salt (PCP-MeI) have also been shown to cause inhibition of acetylcholinesterase and butyrylcholinesterase (3, 4). The antimuscarinic receptor action of PCP and PCP-MeI is demonstrated by their moderate antagonism of ACh-induced contractions of guinea pig ileal smooth muscle (3), by the competitive inhibition of the weak mydriatic action of PCP (3), and by the inhibition of binding of [^{3}H]*N*-methyl-4-piperidyl benzilate

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[1] Present address: Department of Pharmacology, Medical College of Georgia, Augusta, Georgia 30912.

[2] Abbreviations used: CD, *cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide; ACh, acetylcholine; [^{3}H]QNB, [^{3}H](DL)3-quinuclidinyl benzilate; PCP, phencyclidine; PCP-MeI, phencyclidine methiodide.

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(4) and [^{3}H](DL)3-quinuclidinyl benzilate ([^{3}H]QNB) (5, 6) to muscarinic receptors in mammalian brain.

Because of the difficulties inherent in physiological measurements of responses of muscarinic receptors in brain tissue, such studies have been conducted mainly on peripheral receptors in smooth muscle. Muscarinic receptors in both peripheral and central tissues have been identified by biochemical methods. A good correlation was found between the physiologically and biochemically determined affinity constants for several muscarinic drugs, which underscored the reliability of specific [^{3}H]-QNB binding for identification of muscarinic receptors in subcellular preparations (9). The similar drug stereoselectivity of peripheral and central muscarinic receptors suggested comparable structural geometry in the two receptor sites (9).

The present study was initiated to investigate the effects of PCP and PCP-MeI on agonist-induced contraction of smooth muscle of guinea pig ilea and to compare them to the inhibition by PCP of [^{3}H]QNB binding to muscarinic receptors in these ilea and rat brain cerebral cortex and brain stem. The data suggest that PCP and PCP-MeI are antagonists of muscarinic receptors in both tissues.

METHODS

Recording of isotonic contractions. Male albino guinea pigs (350–500 g) were decapitated, and 20 cm of the terminal ileum was removed and placed in Tyrode's solution kept at 37°C. The longitudinal muscle was removed by a method essentially similar to that described by Rang (10). The Tyrode's solution had the following composition (mM): NaCl, 137; KCl, 2.7; $CaCl_2$, 1.8; $MgCl_2$, 0.88; NaH_2PO_4, 0.35; $NaHCO_3$, 12.0; and dextrose, 5.5.

Segments (2 cm) of isolated longitudinal smooth muscle were suspended, fixed at the bottom end, in jacketed glass baths of 10-ml capacity containing oxygenated Tyrode's solution at 37°C. The top of the muscle segment was connected to a light lever with a 9:1 magnification ratio and a resting tension of 300 mg. Responses were recorded on smoked paper. Tissues were initially equilibrated in Tyrode's solution for 60 min with a solution change every 15 min. We used the muscarinic stimulant *cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide (CD) [available in our laboratory (11)] in preference to ACh since it is not hydrolyzed by esterases, is at least as active as ACh as a muscarinic stimulant, and has little or no nicotinic activity. PCP and PCP-MeI were also available in our laboratories (12). Two cumulative dose–response curves to CD were determined at 60-min intervals, and the second was used as a control. Cumulative dose–response curves to CD were then determined in the presence of various concentrations of PCP or PCP-MeI. The antagonists were equilibrated for 10 min prior to the determination of a dose–response curve. Only one antagonist concentration was employed in any tissue. From the dose–response curves, a plot of log (dose ratio-1) against the negative log of the molar concentration of antagonist gave the pA_2 value according to the method of Arunlakshana and Schild (13).

Membrane preparation. Wistar rats (175 to 250 g) were killed by a sharp blow to the upper back. The cerebral cortex (gray and white matter), telencephalon, or brain stem was homogenized in 10 vol of ice-cold 50 mM sodium phosphate buffer, pH 7.2, in a Teflon-glass homogenizer and centrifuged at 1000*g* for 5 min. The resulting supernatant was spun at 40,000*g* for 20 min, and the pellet was suspended in the phosphate buffer and used without further treatment.

Guinea pig ilea for use in the binding studies were obtained on ice from Rockland (Gilbertsville, Pa.) within 4 h of the animal's deaths. The longitudinal muscles were homogenized in 50 mM sodium phosphate buffer, pH 7.2, in a Sorvall Omnimixer for 3 min at the maximum speed, filtered through five layers of cheesecloth, and centrifuged for 5 min at 1000*g*. The supernatant was spun at 40,000*g* for 20 min, and the resulting pellet was rinsed with deionized, glass-distilled water and then suspended in a minimal quantity of water with a Teflon-glass homogenizer. The membranes were frozen in a dry ice–ethanol bath, lyophilized for 12 h, and stored desiccated at −20°C for up to 2 months. For use in binding studies the material was homogenized (10 mg dry weight/ml) in phosphate buffer and used without further treatment.

The protein content of the brain and smooth muscle membranes was determined by the method of Lowry *et al.* (14) using bovine serum albumin as the standard.

Muscarinic receptor binding. This was determined by the binding of tritiated [^{3}H](DL)3-quinuclidinyl benzilate ([^{3}H]QNB) (29.4 Ci/mmol, New England Nuclear), a specific and potent muscarinic antagonist (15). A suspension of the membranes (12.5–50 μg protein/ml) was incubated with [^{3}H]QNB (10^{-11} to 10^{-9} M, as noted) in 50 mM Na_2HPO_4, pH 7.2 (final volume 10 ml, except as noted). After 60 min, the free [^{3}H]QNB was separated from the bound ligand by rapid filtration with suction through Whatman GF/B glass-fiber filters. The radioactivity content of the filters was determined by scintillation counting in 10-ml Filmware (Nalge/Sybron) tubes using a toluene cocktail containing 4% Bio-Solv (Beckman). The counting efficiency in a Beckman 3133-P counter was 40–50%. Nonspecific binding was determined by running a second incubation series which contained 2 μM unlabeled atropine. All points, including those to determine nonspecific binding, were carried out in triplicate.

The interaction of PCP and PCP-MeI with the muscarinic receptors was determined from their ability to inhibit the specific binding of [^{3}H]QNB. PCP binding curves were determined from their inhibition of [^{3}H]QNB binding under conditions such that the concentrations of free [^{3}H]QNB and of muscarinic receptors (10–20 and 2–4 pM, respectively) were as small a fraction of the measured [^{3}H]QNB dissociation constants (60–120 pM in brain, 110–400 pM in ileum) as possible. Receptors in brain stem membranes were present at a concentration less than half, and had an affinity for [^{3}H]QNB 40–60% lower than, those of cerebral cortex membranes. Therefore, larger amounts of protein and [^{3}H]QNB were used when assaying brain stem binding. Moreover, the concentration of free [^{3}H]QNB was adjusted by the amount of binding to the membranes when such a binding would alter the concentration by more than 5%. Using these precautions, the measured affinities of PCP, PCP-MeI,

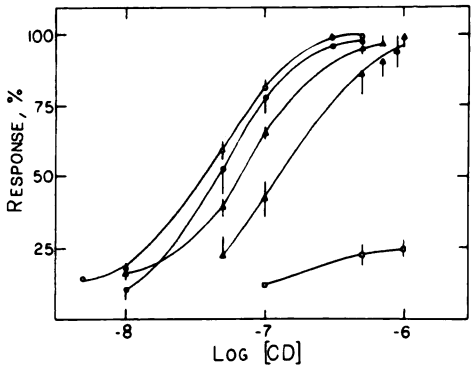

FIG. 1. *Dose-response curves of the longitudinal muscle of guinea pig ileum to CD in the absence* (○) *and presence of PCP, 1 μM* (●), *5 μM* (△), *10 μM* (▲), *and 50 μM* (□)

Each point and vertical line represent the mean of three experiments ± SD.

and unlabeled QNB were not affected by altering the receptor concentration from 2 to 10 pM, suggesting that the measured binding is an accurate reflection of the ligands' association with the receptor (16). Dissociation constants (K_d) were determined from linear regression analyses of the slopes of Scatchard plots of the binding data. Inhibition constants (K_i) were determined from the slopes in double-reciprocal plots of [^{3}H]QNB binding in the presence of various concentrations of drug or, in the case of muscle contraction studies, from parameters of Schild plots as revealed by linear regression analyses.

RESULTS

Muscle contraction. The effects of PCP on CD-induced contractions of guinea pig ileal longitudinal muscle are shown in Fig. 1. The dose-response curves were shifted to the right when PCP was present at concentrations of from 1 to 10 μM. In this range the inhibition was competitive and completely overcome by stimulating the muscle with higher concentrations of CD. However, at 50 μM PCP, there was noncompetitive inhibition of the contraction response that could not be reversed by CD at concentrations of up to 30 μM.

PCP-MeI was slightly more potent as an inhibitor of ileal muscle contraction than PCP, and in contrast to PCP, there was no evidence for noncompetitive interaction with PCP-MeI up to a concentration of 100 μM (Fig. 2). Schild plots depicting the inhibition by PCP and PCP-MeI are presented in Fig. 3. Linear regression analysis gave pA_2 values of 6.7 and 6.35, corresponding to inhibition constants (K_i) of 0.20 and 0.45 μM for PCP-MeI and PCP, respectively (Table 1). The slopes of the lines (1.07 and 0.97, respectively) were close to unity, consistent with competitive inhibition.

Receptor binding. Binding of [^{3}H]QNB to rat telencephalon in the presence of various concentrations of PCP and PCP-MeI is depicted in Figs. 4 and 5. Variability in the amount of nonspecific binding precluded the use of [^{3}H]QNB concentrations above 1.8 nM. PCP-MeI was 3–4.5 times more potent in inhibiting [^{3}H]QNB binding than PCP. Inhibition by PCP and PCP-MeI of [^{3}H]QNB binding was competitive, as shown by the double-reciprocal plots (insets of Figs. 4 and 5). The

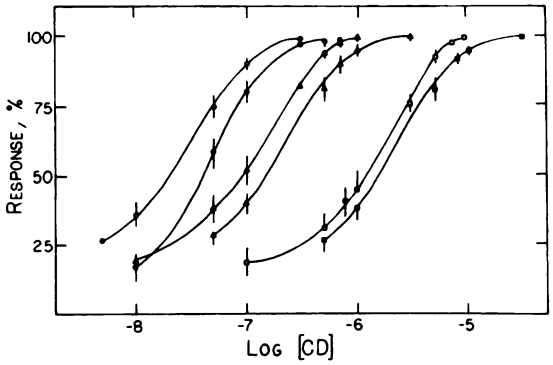

FIG. 2. *Dose-response curves of the longitudinal muscle of guinea pig ileum to CD in the absence* (○) *and presence of PCP-MeI, 1 μM* (●), *5 μM* (△), *10 μM* (▲), *50 μM* (□), *and 100 μM* (■)

Each point and vertical line represent the mean of three experiments ± SD.

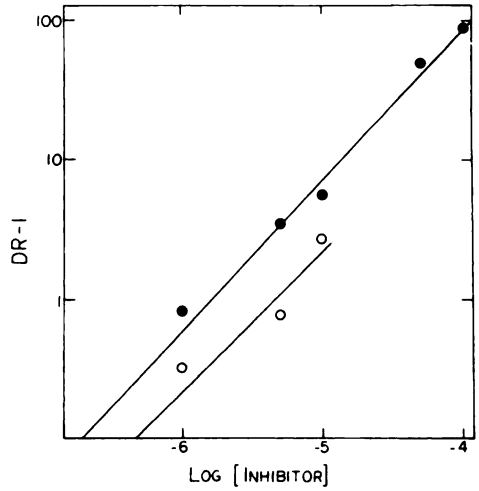

FIG. 3. *Schild plots of the response of the longitudinal muscle of guinea pig ileum to CD in the presence of PCP* (○) *and PCP-MeI* (●)

The data are derived from the information presented in Figs. 1 and 2. DR, the ratio of the concentrations of CD which produce 50% of maximum muscle contraction in the presence vs the absence of the inhibitor (PCP and PCP-MeI). Linear regression analyses indicate pA_2 values of 6.35 and 6.7 for PCP and PCP-MeI. The slopes of the lines (0.97 and 1.07, respectively) do not deviate significantly from unity.

TABLE 1

Binding constants of PCP and PCP-MeI for muscarinic acetylcholine receptors

Compound	Tissue	K_i[a] (N)	K_d[b] (N)	K_i[c]
		μM	μM	μM
PCP	Cerebral cortex	1.4 ± 0.7 (4)	2.5 ± 0.5 (3)	
	Brain stem		3.7 ± 0.4 (3)	
	Ileum	1.6 ± 0.9 (3)		0.45
PCP-MeI	Cerebral cortex	0.4 ± 0.1 (4)	0.68 ± 0.02 (3)	
	Brain stem		0.68 ± 0.03 (4)	
	Ileum	0.8 ± 0.4 (3)		0.20

[a] Inhibition constants determined from double-reciprocal plots of [^{3}H]QNB binding in the presence of various PCP concentrations.

[b] Binding dissociation constants determined from the slopes of Scatchard plots of PCP binding inferred from their inhibition of [^{3}H]QNB binding as described in Methods.

[c] K_i is the negative antilogarithm of the pA_2 value determined from Schild plots of CD-induced smooth muscle contraction in the presence of PCP or PCP-MeI.

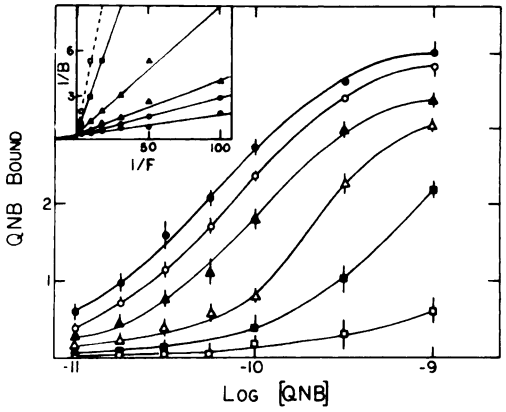

FIG. 4. *Binding of [^{3}H]QNB to muscarinic receptors in membranes from rat telencephalon in the absence (●) and presence of the following concentrations of PCP: 1 μM (○), 3.2 μM (▲), 10 μM (△), 32 μM (■), and 100 μM (□)*

Each point and vertical line represent the mean ± SD of three experiments. QNB bound is in pmol/mg protein, and QNB concentration is in M. The inset represents a double-reciprocal plot of the data. B, amount bound in pmol/mg protein. F, free [^{3}H]QNB concentration in nM. Binding values obtained at high concentrations of [^{3}H]QNB (>0.32 nM) are omitted for clarity, and certain data points are off scale. Lines are drawn from least-squares linear regression analyses of all the data. The dashed lines represent binding in the presence of 100 μM PCP; binding was detected only at the three highest concentrations of [^{3}H]QNB.

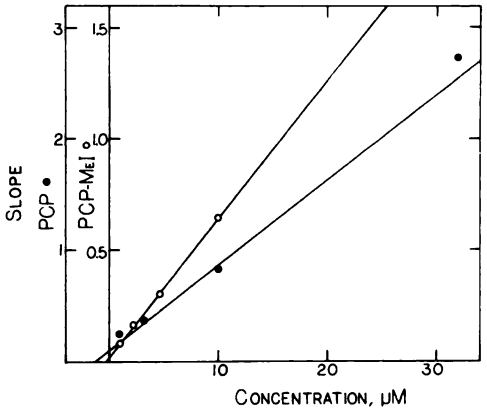

FIG. 6. *The slopes of the double-reciprocal plots in the insets to Figs. 4 and 5 as a function of the PCP (●) or PCP-MeI (○) concentration*

These functions are linear ($r > 0.99$). The indicated inhibition constants (X intercepts) are 1.4 and 0.38 μM with PCP and PCP-MeI, respectively.

slopes of the double-reciprocal plots are depicted as a function of the concentration of PCP or PCP-MeI in Fig. 6. These relationships were linear, indicating competitive inhibition with K_i values (obtained from the X-axis intercepts) of 1.4 ± 0.7 and 0.38 ± 0.1 μM for PCP and PCP-MeI, respectively (Table 1). Scatchard plots of the same data analyzed as PCP binding (not shown) are linear ($r > 0.95$) and indicate the same K_i at every PCP concentration, emphasizing that PCP and [^{3}H]QNB bound to muscarinic ACh receptors in a mutually exclusive manner. Moreover, maximal [^{3}H]QNB binding did not vary significantly when binding was measured in the presence of various concentrations of PCP.

The binding of PCP (Fig. 7) and PCP-MeI (Fig. 8) to both cortical and brain stem receptors followed a law of mass action relationship, with no obvious indications of cooperative interactions or multiple receptor populations. Hill plots of the binding data yielded slopes not significantly different from one. The dissociation constant (K_d) obtained from linear regression analyses of PCP binding to cerebral cortex receptors (2.5 ± 0.5 μM) was significantly different from that to brain stem receptors (3.7 ± 0.4 μM). The K_d values of PCP-MeI to muscarinic receptors were the same for cortex and brain stem (K_d = 0.68 ± 0.02 and 0.68 ± 0.03 μM, respectively). Binding affinities of cerebral cortex receptors for PCP and PCP-MeI determined in these experiments were twofold less than those determined in experiments in which the binding of several concentrations of [^{3}H]QNB in the presence of a single concentration of the competing phencyclidine was measured (Table 1, compare K_i and K_d values). These discrepancies are evident despite the selection of assay conditions which should yield accurate binding constants. The reason for these discrepancies is unknown.

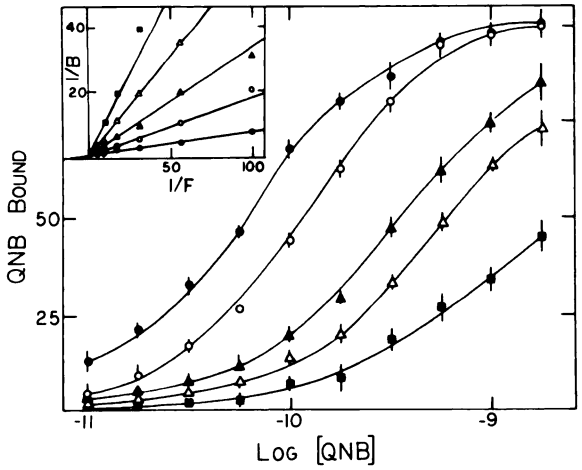

FIG. 5. *Binding of [^{3}H]QNB to muscarinic receptors in membranes from rat telencephalon in the absence (●) and presence of the following concentrations of PCP-MeI: 1 μM (○), 2.2 μM (▲), 4.6 μM (△), and 10 μM (■)*

Each point and vertical line represent the mean ± SD of three experiments. QNB bound is in pmol/mg protein, and QNB concentration is in nM. The inset represents a double-reciprocal plot of the data. B, amount bound in pmol/mg protein. F, free [^{3}H]QNB concentration in nM. Binding values at several of the highest [^{3}H]QNB concentrations are not visible, and a few data points are off scale. Lines are drawn from linear regression analyses of all the data.

The inhibition of 0.1 μM [^{3}H]QNB binding to muscarinic receptors in the longitudinal muscle of guinea pig ileum by PCP is shown in Fig. 9. QNB binding was reduced with an ID_{50} of about 10 μM. Double-reciprocal plots of [^{3}H]QNB binding to ileum muscarinic receptors in the presence of two concentrations of PCP are presented in Fig. 10. PCP was a competitive inhibitor of QNB binding with an apparent K_i of 1.6 ± 0.9 μM (range of values from six determinations on three muscle preparations). PCP-MeI inhibited QNB binding with an apparent K_i of 0.8 ± 0.4 μM (Table 1). The K_d for QNB binding was significantly greater with ileum receptors

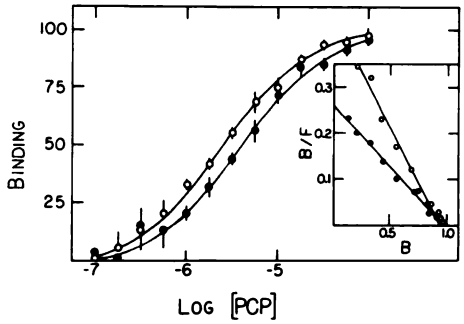

FIG. 7. *The binding of PCP to brain muscarinic receptors in membranes from rat cerebral cortex* (○) *and brain stem* (●) *as a function of the PCP concentration in* M

Binding, expressed as percentage of maximal binding, was determined from the inhibition of 10 (cortex) and 20 pM (brain stem) [^{3}H]-QNB at a receptor concentration of 4 (cortex) or 2 pM (brain stem). The inset is a Scatchard plot of the data (lines drawn from linear regression analyses). B, percentage of maximal binding. F, free [^{3}H]-QNB concentration in nM. Because of the large variability in binding measures representing fractional receptor occupancies of less than 10%, these data were omitted from the Scatchard analyses.

(300 ± 125 pM, N = 4) compared to neural receptors (90 ± 20 pM, N = 8) in these experiments.

Treatment of membranes from rat cerebral cortex and the longitudinal muscle of guinea pig ileum with 1 mM N-ethylmaleimide for 20 min at 37°C did not affect the inhibition of [^{3}H]QNB binding to muscarinic receptors by PCP.

DISCUSSION

PCP and PCP-MeI interact with muscarinic receptors as shown by their inhibition of specific [^{3}H]QNB binding to brain (Figs. 4–6) and ileal muscle (Figs. 9 and 10) as well as by the inhibition of CD-induced contraction of ileal smooth muscle (Figs. 1–3). PCP and PCP-MeI differ in one important aspect: PCP-MeI is positively charged at all times. This greatly reduces its accessibility to hydrophobic membrane regions and its ability to cross biomembranes and may well explain differences in the effects of the two drugs on muscarinic systems. CD is a potent and specific muscarinic agonist, which has an

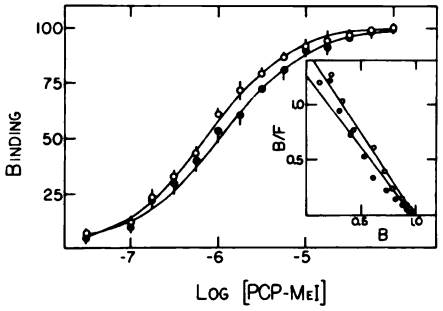

FIG. 8. *The binding of PCP-MeI to brain muscarinic receptors in membranes from rat cerebral cortex* (○) *and brain stem* (●) *as a function of the PCP-MeI concentration in* M

Binding is expressed as percentage of maximum. Ligand and receptor concentrations were as indicated in Fig. 7. The inset is a Scatchard plot drawn from a linear regression of all data representing more than 10% receptor occupancy. B, pmol bound/mg protein. F, PCP concentration in nM.

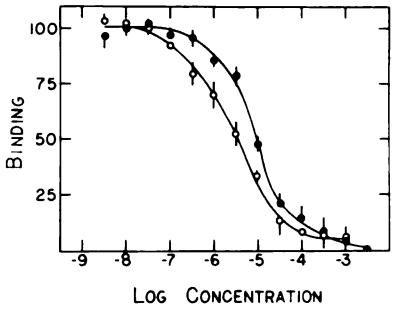

FIG. 9. *Inhibition of [^{3}H]QNB binding to muscarinic receptors in longitudinal muscles of guinea pig ileum by PCP* (●) *and PCP-MeI* (○)

The indicated concentrations of phencyclidines were incubated with muscle membranes and 0.1 nM [^{3}H]QNB in a final volume of 2 ml. Binding is expressed as percentage of maximal; concentrations are in M.

extremely high intrinsic activity, and is capable of causing half of maximal muscle contraction when present at concentrations at which it occupies only a small fraction of the [^{3}H]QNB-identified receptor binding sites (9). The inhibition by PCP of CD-induced muscle contraction has a dual nature, since at concentrations at and below 10 μM the inhibition is competitive, while noncompetitive inhibition is observed at 50 μM (Fig. 1). On the other hand, the inhibition by PCP-MeI is competitive at the highest concentrations of PCP-MeI tested (100 μM) (Fig. 2). By comparison, both PCP and PCP-MeI are competitive inhibitors of [^{3}H]QNB binding to brain receptors at all concentrations tested (up to 1 mM) (Figs. 4 and 5). Scatchard plots reveal only one component to the binding, and Hill plots are linear with slopes of one. Thus, there are no indications of multiple or cooperative interactions with regard to PCP binding to the muscarinic receptor population identified by [^{3}H]QNB binding. These findings are true with muscarinic receptors from rat cerebral cortex and brain stem, as well as from guinea pig ileal muscle (Figs. 7 and 9). The noncompetitive inhibition of muscle contraction seen at high concentrations of PCP, which is not observed in the binding studies, may be a reflection of an interaction of PCP with a site

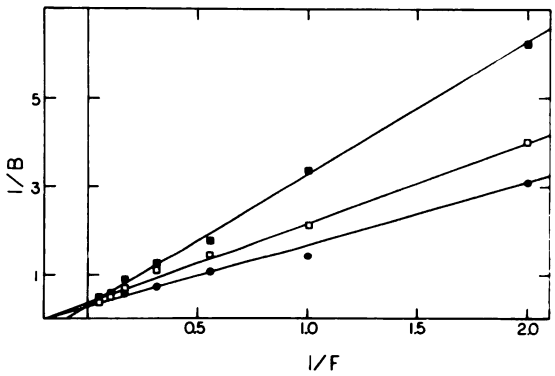

FIG. 10. *Double-reciprocal plot of [^{3}H]QNB binding to muscarinic receptors in longitudinal muscle of guinea pig ileum in the absence* (●) *and presence of 1* (□) *and 10* (■) μM *PCP*

Binding was carried out in 10 ml of incubation medium containing 4 pM receptor. The lines are drawn using linear regression analyses. B, amount bound in pmol/mg protein. F, free [^{3}H]QNB concentration in nM.

or sites that affect the muscle contraction apparatus that is unrelated to the muscarinic receptor binding site. Since the noncompetitive effect is seen only with PCP, and not with PCP-MeI, it is possible that the site(s) is intracellular or involves significant partitioning into the membranes.

We have previously reported close correlations between the ability of receptor antagonists and weak agonists to inhibit [^{3}H]QNB binding to guinea pig ileum longitudinal muscle and their ability to inhibit or stimulate contraction of these muscles (9), and the present data on PCP and PCP-MeI are in reasonable agreement with these correlations (Figs. 1, 2, and 10). However, the K_i values for inhibition of muscle contraction are about fourfold smaller than those of QNB binding (Table 1). Still, the good agreement in the ratio of potency of PCP to PCP-MeI in inhibiting both muscle contraction and QNB binding suggests an interaction at equivalent molecular sites.

Pharmacologically effective doses of PCP produce concentrations in the brain of several micromolar (4). The inhibition constants of specific [^{3}H]QNB binding in brain and ileum by PCP and PCP-MeI fall within this range since they are 0.4 to 1.6 μM, respectively (Table 1). Their affinities are quite low when compared to most known antagonists of muscarinic receptors, which are 100- to 1000-fold more potent (9).

It is difficult to determine whether a competitive muscarinic drug acts as an agonist or an antagonist in the brain. Binding studies by themselves indicate only whether or not the compound can occupy the receptor's ligand recognition site. Yet three binding properties have now emerged which appear to be characteristic of either agonist or antagonist binding to brain muscarinic receptors. One is the heterogeneity found in the affinities that muscarinic receptors in different parts of the brain have for agonists, but not antagonists (17, 18). Thus, brain stem muscarinic receptors have up to 100-fold greater affinities than cortical receptors for agonists, while their affinities for [^{3}H]QNB, atropine, and scopolamine are the same (17). PCP and PCP-MeI have similar affinities for muscarinic receptors of brain stem and cerebral cortex, thus behaving more like antagonists (Table 1). The second criterion is the multiple affinities for agonists and the single affinity for antagonists observed in the binding to muscarinic receptors in all brain regions (19). PCP and PCP-MeI binding to brain or muscle receptors is with a single affinity as for antagonists (Figs. 4, 5, 9, and 10). The third criterion is the increase in affinity for agonists, but not antagonists, which occurs upon reductive alkylation of neural membranes with 1 mM *N*-ethylmaleimide without much change in affinity for muscarinic receptor antagonists (20). We now demonstrate that *N*-ethylmaleimide treatment does not alter the affinity of PCP for cerebral cortex receptors. Therefore, according to these criteria, the binding of PCP and PCP-MeI to brain receptors exhibits properties associated with muscarinic antagonists and not agonists.

It is clear that PCP and PCP-MeI interact with muscarinic receptors. Their affinities for these receptors are within the range of concentrations that can be reached in the brain and rest of the body. However, as shown by the data of the previous two papers, muscarinic receptors are evidently not the only sites of interaction.

REFERENCES

1. Tsai, M. -C., E. X. Albuquerque, R. S. Aronstam, A. T. Eldefrawi, M. E. Eldefrawi and D. J. Triggle. Sites of action of phencyclidine. I. Effects on the electrical excitability and chemosensitive properties of the neuromuscular junction of skeletal muscle. *Mol. Pharmacol.* **18:** 159–166 (1980).
2. Albuquerque, E. X., M. -C. Tsai, R. S. Aronstam, A. T. Eldefrawi and M. E. Eldefrawi. Sites of action of phencyclidine. II. Interaction with the ionic channel of the nicotinic receptor. *Mol. Pharmacol.* **18:** 167–183 (1980).
3. Maayani, S., H. Weinstein, N. Ben-Zvi, S. Cohen and M. Sokolofsky. Psychotomimetics as anticholinergic agents. I. 1-Cyclohexylpiperidine derivatives: Anticholinesterase activity and antagonist activity to acetylcholine. *Biochem. Pharmacol.* **23:** 1263–1281 (1974).
4. Kloog, Y., M. Rehavi, S. Maayani and M. Sokolovsky. Anticholinesterase and antiacetylcholine activity of 1-phenylcyclohexylamine derivatives. *Eur. J. Pharmacol.* **45:** 221–227 (1977).
5. Vincent, J. P., D. Cavey, J. M. Kamenka, P. Geneste and M. Lazdunski. Interactions of phencyclidines with the muscarinic and opiate receptors in the central nervous system. *Brain Res.* **152:** 176–182 (1978).
6. Jim, K., D. J. Triggle, E. X. Albuquerque, R. S. Aronstam and M. E. Eldefrawi. Phencyclidine interaction with peripheral and central muscarinic receptors. *Fed. Proc.* **38:** 274 (1979).
7. Maayani, S. and H. Weinstein. Some structure activity relationships of phencyclidine derivatives as anticholinergic agents in vitro and in vivo, in *Membrane Mechanisms of Drugs of Abuse* (C. W. Sharp and L. G. Abood, eds.). Alan R. Liss, New York, 91–106 (1979).
8. Weinstein, H., S. Maayani, S. Srebenik, S. Cohen and M. Sokolovsky. Psychotomimetic drugs as anticholinergic agents. II. Quantum mechanical study of molecular interaction potentials of cyclohexylpiperidine derivatives with the cholinergic receptor. *Mol. Pharmacol.* **9:** 820–834 (1973).
9. Aronstam, R. S., D. J. Triggle and M. E. Eldefrawi. Structural and stereochemical requirements for muscarinic receptor binding. *Mol. Pharmacol.* **15:** 227–234 (1979).
10. Rang, H. P. Stimulant actions of volatile anesthetics on smooth muscle. *Br. J. Pharmacol.* **22:** 356–365 (1964).
11. Chang, K. J., R. C. Deth and D. J. Triggle. Structural parameters determining cholinergic and anticholinergic activities in a series of 1,3-dioxolanes. *J. Med. Chem.* **15:** 243–247 (1972).
12. Kalir, A., H. Edery, Z. Pelah, D. Balderman and G. Porath. 1-Phenylcycloalkylamine derivatives. II. Synthesis and pharmacological activity. *J. Med. Chem.* **12:** 473–477 (1969).
13. Arunlakshana, O., and H. O. Schild. Some quantitative uses of drug antagonists. *Br. J. Pharmacol.* **14:** 48–58 (1959).
14. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275 (1951).
15. Yamamura, H. I., and S. H. Snyder. Muscarinic cholinergic binding in rat brain. *Proc. Natl. Acad. Sci. USA* **71:** 1725–1729 (1974).
16. Chang, K. -J., S. Jacobs and P. Cuatrecasas. Quantitative aspects of hormone-receptor interactions of high affinity. Effect of receptor concentration and measurement of dissociation constants of labeled and unlabeled hormones. *Biochim. Biophys. Acta* **406:** 294–303 (1975).
17. Aronstam, R. S., C. Kellogg and L. G. Abood. Development of muscarinic cholinergic receptors in inbred strains of mice: Identification of receptor heterogeneity and relation to audiogenic seizure susceptibility. *Brain Res.* **162:** 231–241 (1979).
18. Kloog, Y., Y. Egozi and M. Sokolovsky. Characterization of muscarinic acetylcholine receptors from mouse brain: Evidence for regional heterogeneity and isomerization. *Mol. Pharmacol.* **15:** 545–558 (1979).
19. Birdsall, N. J. M., A. S. V. Burgen and E. C. Hulme. The binding of agonists to brain muscarinic receptors. *Mol. Pharmacol.* **14:** 723–736 (1978).
20. Aronstam, R. S., L. G. Abood and W. P. Hoss. Influence of sulfhydryl and heavy metals on the functional state of the muscarinic acetylcholine receptor in rat brain. *Mol. Pharmacol.* **14:** 575–586 (1978).

Send reprint requests to: Mohyee E. Eldefrawi, Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, Md. 21201.