SPECIFIC BINDING OF [^{3}H]PHENCYCLIDINES TO MEMBRANE PREPARATION

Possible interaction with the cholinergic ionophore

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1. Introduction

We have suggested that the antinicotinic effect of phencyclidine and its derivatives is due to their direct interaction with the cholinergic ionophore, or to their interference with the coupling mechanism between the receptor site and the ionophore [1]. These suggestions and those in [2,3], have been deduced from physiological and competition experiments. We therefore decided to investigate the nature of this interaction by means of direct binding experiments using the ^{3}H-labeled derivatives. The phencyclidines exhibit multiple interactions ([1] and literature cited therein); their specific binding was studied in *Torpedo* electric organ since this enabled us to focus on the nicotinic cholinergic system (reviewed [4]).

2. Experimental

Torpedo electric organs were homogenized and fractionated as in [5,6]. The P_2 pellet was resuspended in the reaction buffer so that each ml contained 1.2–1.4 g original organ. Binding was determined by the centrifugation method [7] in a buffer containing 250 mM NaCl, 5.0 mM KCl, 2.0 mM $MgCl_2$, 4.0 mM $CaCl_2$ and 10 mM Na-phosphate (pH 7.4).

The reaction mixture contained 50 μl membrane preparation, 50 μl buffer containing [^{3}H]phencyclidine derivative and either 100 μl reaction buffer, or 50 μl buffer and 50 μl buffer containing unlabeled ligand. Following incubation for 15 min (after which time the reaction is at equilibrium) the tubes were centrifuged for 4 min (Beckman microfuge B); the supernatant was then sampled for determination of free ligand and discarded. The pellets were washed rapidly with 1 ml ice-cold buffer, the tip of the tube was cut and its contents collected in scintillation vials containing 4 ml scintillation liquid (Hydro-Luma, Lumac Systems), stirred with a vortex and kept at room temperature for 24 h. Counting was carried out with a Packard Prias PL, with a counting efficiency of 40%.

Phencyclidine (1-(1-phenylcyclohexyl)piperidine) and 3-aminophencyclidine (1-(1-(3-aminophenyl)-cyclohexyl)piperidine) were prepared as in [8]. The amino analog was previously described as a 4-amino analog but recent studies [9] have established that this derivative is the 3-aminophencyclidine. [^{3}H]Phencyclidine (23.7 Ci/mmol) and 3-[^{3}H]aminophencyclidine (26.9 Ci/mmol) were prepared and tested for purity (>96%) as in [8]. For binding assays the labeled drugs were isotopically diluted (1:10) with unlabeled drugs. ^{125}-Labeled α-bungarotoxin (^{125}I-αBgt) (141.2 Ci/mmol) was purchased from NEN.

The nicotinic acetylcholine receptor was assayed with ^{125}I-αBgt by the method in [10] and protein was determined according to [11].

3. Results and discussion

The total binding of [^{3}H]phencyclidine to the membrane preparation of *Torpedo* electric organ (fig.1A) is described by a curvilinear binding isotherm which can be resolved into two binding components: a linear non-saturable binding (which can be separately determined in the presence of 10^{-3} M unlabeled

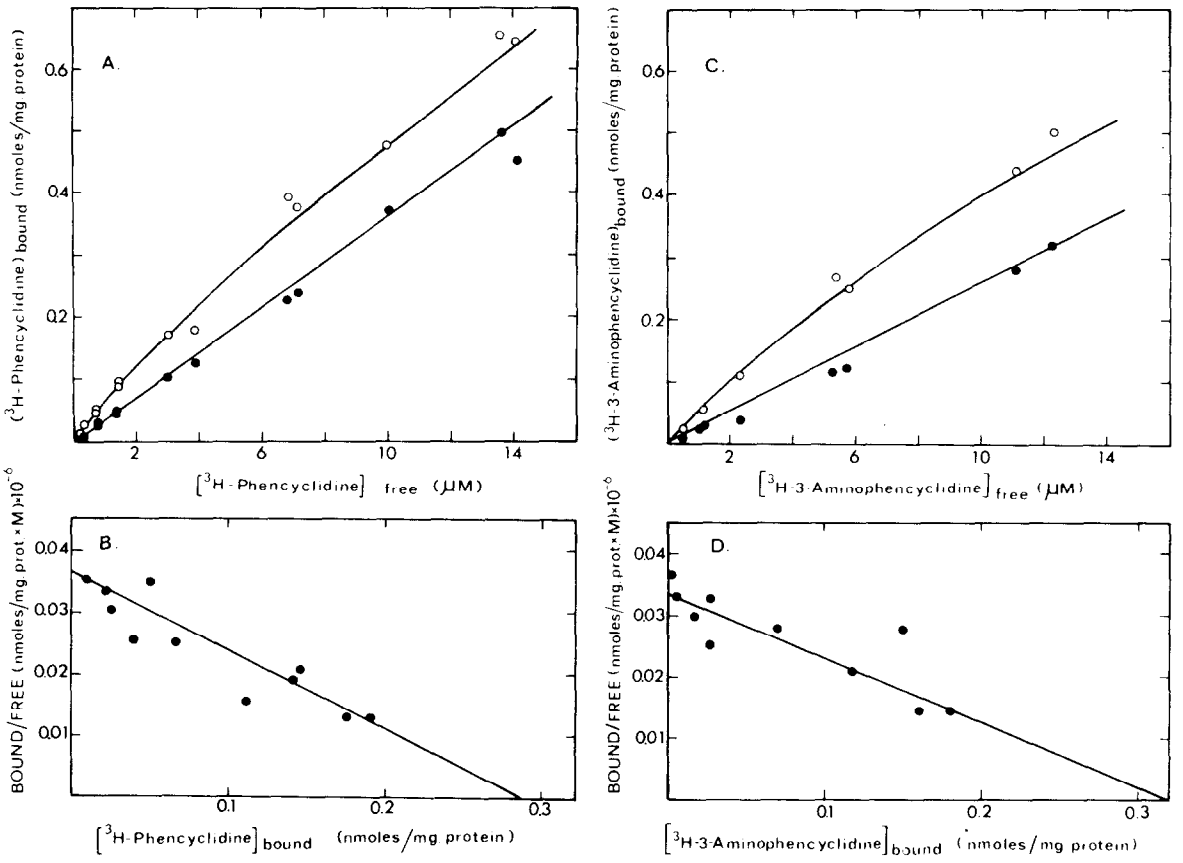

Fig.1. Binding of [^{3}H]phencyclidine and 3-[^{3}H]aminophencyclidine to membrane preparation of *Torpedo* electric organ at 25°C. Binding was determined as in section 2. Each point is the mean of triplicate samples whose standard error was <15%. (A) Binding of [^{3}H]phencyclidine in the presence (•) or absence (○) of 10^{-3} M phencyclidine. (B) Scatchard plot of the specific component of binding (data from (A)). (C) Binding of 3-[^{3}H]aminophencyclidine in the presence (•) and absence (○) of 10^{-3} M 3-aminophencyclidine. (D) Scatchard plot of the specific components of binding (data from (C)).

phencyclidine or 3-aminophencyclidine; see fig.1A), superimposed on a hyperbolic saturable binding isotherm, calculated by subtracting the linear binding from the total binding (fig.1A). The saturable binding occurring in the μM range, when replotted according to [12], yielded linear curves (fig.1B), suggesting that [^{3}H]phencyclidine binds to a single class of non-interacting binding sites, (K_d 7.5 ± 2.5 μM; binding capacity = 0.29 ± 0.03 nmol/mg protein). In the same preparation, binding site capacity for ^{125}I-α-Bgt was 1.1 nmol/mg protein. Hence the ratio of [^{3}H]phencyclidine to ^{125}I-αBgt binding is ~1:4. Similar results were obtained with the second analog studied, 3-[^{3}H]aminophencyclidine (fig.1B,C). The K_d for the latter, 15.5 ± 4.5 μM, was twice as high as that determined for [^{3}H]phencyclidine, but the binding capacity was similar (0.32 ± 0.04 nmol/ mg protein). The saturable binding component of both [^{3}H]phencyclidine and 3-[^{3}H]aminophencyclidine could be inhibited by unlabeled phencyclidine, 3-aminophencyclidine, or the local anesthetic procaine (fig.2A,B). Dissociation constants of the unlabeled phencyclidine derived from inhibition experiments in which a simple competitive interaction was assumed are in good agreement with those obtained from the direct binding (table 1). Cholinergic ligands such as αBgt, *d*-tubocurarine and carbamylcholine, which are known to bind to the nicotinic receptor, and those which are known to bind to the muscarinic receptor such as atropine and *N*-methyl-piperidyl benzilate, did not affect the saturable binding component of the labeled phencyclidines at 50–1000 μM (table 1).

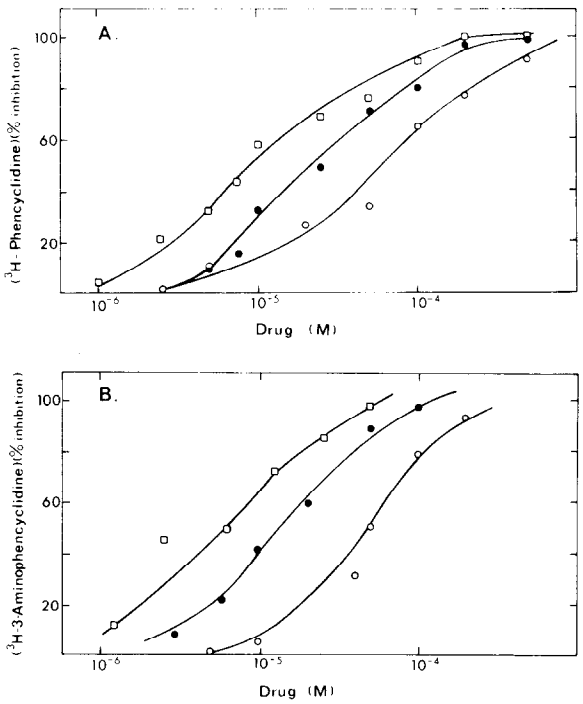

Fig.2. Inhibition of [^{3}H]phencyclidine and 3-[^{3}H]aminophencyclidine binding. Aliquots (50 μl) of membrane preparation were incubated with 0.65 μM [^{3}H]ligand and with the unlabeled ligand at the concentrations indicated. Binding was determined in triplicate as in section 2. Data are presented as % inhibition of the specific component of the [^{3}H]ligand binding by phencyclidine (□), 3-aminophencyclidine (●) and procaine (○). (A) Inhibition of [^{3}H]phencyclidine binding. (B) Inhibition of 3-[^{3}H]aminophencyclidine binding.

Phospholine-iodide (50 μM), a cholinesterase inhibitor, did not affect the binding of the [^{3}H]phencyclidines.

Hence, we conclude that the phencyclidine derivatives under investigation bind at the μM level to specific binding sites present in the *Torpedo* membrane preparation which are not the αBgt binding sites of the nicotinic receptors present in this preparation [4,6,15]. These data are in good agreement with the findings [1–3] that at μM levels phencyclidines do not block binding of ^{125}I-αBgt.

The similar binding capacities for [^{3}H]phencyclidine and 3-[^{3}H]aminophencyclidine, the competitive nature of the binding of labeled and unlabeled phencyclidines, and the good agreement between the K_d values of the labeled and unlabeled drugs, suggest that they bind to the same binding sites. The nature of the specific binding site, i.e., the 'receptor' for phencyclidine, remains in question. However, the likelihood that the ligand receptor for the phencyclidine derivatives is indeed the cholinergic ionophore [13–15], is supported by the following:

(i) Procaine inhibited the specific phencyclidine binding. Procaine and other local anasthetics are known to interact with the cholinergic ionophore and with the acetylcholine binding site [16,17]. However, unlike local anasthetics [17], phencyclidine binding (capacity and affinity) is not affected by the presence of carbamylcholine (50 μM). Thus, under these circumstances the

Table 1
Dissociation constants evaluated from direct binding and competition experiments with [^{3}H]phencyclidine and 3-[^{3}H]aminophencyclidine

Drug	K_d[a] (μM) Direct binding	K_i[b] (μM) Inhibition of [^{3}H]phencyclidine	K_i[b] (μM) Inhibition of 3-[^{3}H]-aminophencyclidine
Phencyclidine	7.5 ± 2.5	8.5 ± 1.5	6.5 ± 2.1
3-Aminophencyclidine	15.5 ± 4.5	19.2 ± 5.3	14.1 + 2.6
Procaine	–	61.5 ± 15.0	54.4 ± 12
α-Bgt	–	> 100	> 100
d-Tubocurarine	–	>1000	–
Carbamylcholine	–	>1000	>1000
Atropine	–	> 100	> 100
4-*N*-Methyl piperidyl benzilate	–	> 100	–

[a] K_d is evaluated from the Scatchard plots as described in fig.1. The mean values ±SD of 5 expt are given

[b] K_i is evaluated from inhibition experiments as shown in fig.2, according to the equation $K_i = I_{50}/(1 + D/K_d)$, where I_{50} is the concentration which inhibits 50% of the specific binding of the labeled phencyclidine derivatives at concentration D. K_d is the dissociation constant of the labeled drug. The mean values ±SD of 3 expt are given

occupation of acetylcholine binding site by cholinergic agonist does not regulate the binding of phencyclidine and lends further support for the specificity of phencyclidine interaction;

(ii) Preliminary results indicated that phencyclidines compete for the histrionicotoxin binding sites [3];

(iii) The ratio of [^{3}H]phencyclidine to ^{125}I-αBgt binding (1:4) is similar to that reported for the specific ionophore toxin (H_{12}HTX) to αBgt binding [15];

(iv) The structural similarity of phencyclidine and histrionicotoxin, as we have pointed out [1].

Finally, in view of the fact that the K_d values reported here for phencyclidine derivatives correlate very well with their anticholinergic potencies [8,18,19] and since these values are at μM levels (where they do not affect the αBgt binding and vice versa) it is strongly suggested that the antinicotinic effect of phencyclidines stems from their interaction with the ionophore system. Thus, radiolabeled as well as fluorescent derivatives of phencyclidine might serve as important tools in further experiments to detect and evaluate, for example, the interaction of other toxins (see [20]), and the ionophore conformation (e.g., buffer effect – open and closed channels).

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