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PRELIMINARY COMMUNICATION

FUNCTIONAL EVIDENCE FOR A SECOND BINDING SITE OF NICOTINIC ANTAGONISTS USING PHENCYCLIDINE DERIVATIVES

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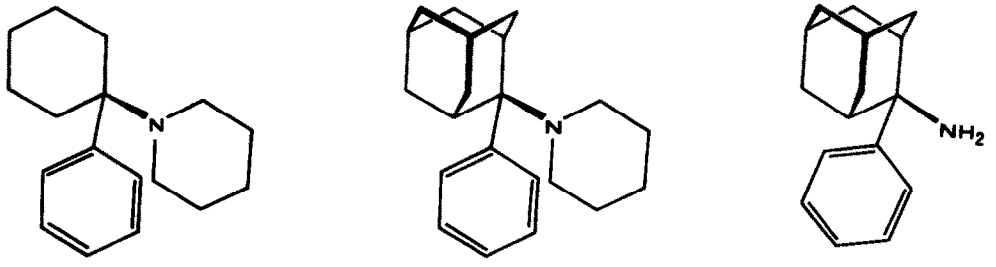
We have previously noted that the antiacetylcholine activity of phencyclidine and its derivatives in the striated muscle from the frog rectus abdominis, is concentration dependent, i.e. at concentration close to the K_D, they induce a parallel shift of the acetylcholine (AcCh) dose response curve, while at concentrations higher than the apparent K_d they induce a non-parallel shift with a concomitant decrease in the maximal response to AcCh (1) (2). Thus, the data indicate that the blocking effect of phencyclidines could not be solely attributed to a simple competition with acetylcholine on the binding sites of the latter. In order to determine whether these drugs interact directly with the cholinergic binding site on the AcCh receptor we have examined the effect of phencyclidine and two new derivatives, namely compounds 2 and 3 (Fig. 1) on the binding of [^{125}I]α-Bungarotoxin ([^{125}I]αBtx) to the nicotinic receptor and compared it to their blocking effect on the isolated striated muscle preparation.

Nicotinic blocking effects were tested on frog rectus abdominis as described previously (1). Binding of [^{125}I]αBtx was studied on crude synaptosomal membrane preparation obtained from the electric organ of Torpedo ocellata (3) by the method of Vogel and Nirenberg (4), and on the same preparation solubilized with 0.5% Triton X-100 and performed according to Klett et al. (5). Synthesis of drugs 2 and 3 is described elsewhere (6). ([^{125}I]αBtx (S.A. 300,000 cpm/nmole) was kindly supplied by Dr. Y. Dudai, The Weizmann Institute, Israel).



1 2 3

Figure 1 - Chemical structure of phencyclidine (1-(1-phenylcyclohexyl) piperidine (1), 1-(2-phenyl-2-adamantanyl) piperidine (2), 2-phenyl-2-adamantamine (3).

The effect of phencyclidine and 2 on the binding of [^{125}I]αBtx to the solubilized nicotinic receptor from Torpedo ocellata is shown in Fig. 2. Inhibition of toxin binding is apparent only at concentration ranges of 10^{-4} - 10^{-3} M, but in all cases it does not exceed 50%. Similar results were observed with the other derivatives investigated (Table 1), while classical nicotinic antagonist like d-tubocurarine desplaced most of the αBtx with a value of I_{50} (Table 1) which is in agreement with values reported previously (7 and references therein). Similarly, binding of [^{125}I]αBtx to the membrane preparation was also affected by the drugs tested only at concentrations larger than 10^{-4} M.

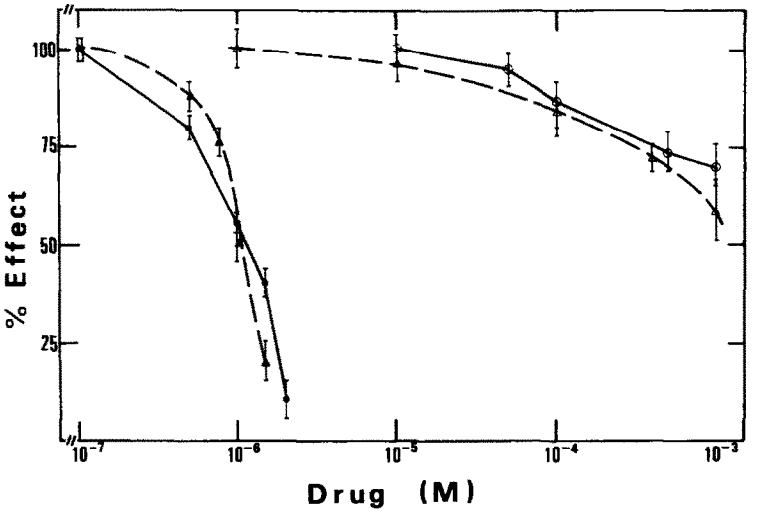


Figure 2 - Antagonism of phencyclidine and compound 2 to the contractile response of frog rectus abdominis to acetylcholine, and their effect on [^{125}I]αBtx binding to solubilized receptor of Torpedo ocellata. [^{125}I]αBtx binding sites concentration was 0.8 nM. Each point represents the mean of 3 separate determinations. Vertical bars denote S.D. Ordinate: % response of the contraction induced by 2 μM AcCh in the presence of phencyclidine (●) and compound 2 (▲) and % of binding of 2.5 nM [^{125}I]αBtx in the presence of phencyclidine (o) and 2 (Δ).

In agreement with previous observations (1) phencyclidine and the derivatives investigated here, exhibit high potency in blocking the effects of cholinergic agonists on the striated muscle, (Fig. 2 and Table 1). Thus, at 1.2 μM phencyclidine and compound 2 inhibit 50% of the contraction induced by 2 μM AcCh. 2-amantadine and 1-phenylcyclohexylamine were approximately 10 times less potent (Table 1). The blocking effect could not be described by a simple competition with AcCh.

However, high potency of phencyclidine derivatives is manifested at concentrations where they do not affect [^{125}I]αBtx binding to the Torpedo cholinergic receptor. This can be explained in at least two ways: 1) non identity of the receptors in these preparations;

Table 1

Drug	Antiacetylcholine activity (Kapp, M)†	Inihibition of [^{125}I]αBtx binding (I_{50}, M)††
Phencyclidine	1.2×10^{-6}	$> 10^{-3}$
1-Phenylcyclohexylamine	1.5×10^{-5}	$> 10^{-3}$
compound 2	1.2×10^{-6}	$> 10^{-3}$
compound 3	3.9×10^{-6}	$> 10^{-3}$
1-amantadine	-	$> 10^{-3}$
2-amantadine	1.1×10^{-5}	$> 10^{-3}$
d-tubocurarine	1.9×10^{-7}	3.3×10^{-7}

† Kapp = Drug concentration which inhibits 50% of the frog rectus abdominis contraction induced by 2 μM acetylcholine.

†† I_{50} drug concentration which inhibits 50% of 2.5 nM [^{125}I]αBtx binding to solubilized preparation of Torpedo ocellata electric organ.

2) phencyclidine and its derivatives exert their antinicotinic activity at a site distinct from the toxin binding site. The cholinergic receptors in striated muscle and electric organs both bind α-Btx and have similar nicotinic profile as judged by physiological and biochemical studies (7-10). Hence the first explanation is probably not valid. Consequently, the second explanation seems more likely. Analogous phenomena have been observed previously with local anesthetics (11) histrionicotoxins (12, 13) and 1-amantadine (14). Hence in analogy (8) (13, 14) the blocking effect of phencyclidines may be due to their direct interaction with the cholinergic ionophore. Alternatively, they may interfere with the coupling mechanism between the receptor site and the ionophore (11) (15). Therefore phencyclidine and its derivatives might serve as a tool to detect and identify such interactions. Further work to establish these hypotheses is currently in progress. It is noteworthy that phencyclidines possess structural similarity to e.g. histrionicotoxin, while compound 2 contains the amantadine residue. Studies carried out in this laboratory have also shown that compounds 2 and 3 have muscarinic-type action in addition to the nicotinic effects described here (to be published). However, the muscarinic effects of these compounds as well as of other phencyclidine derivatives (1) are strictly competitive and the affinity constants determined by binding experiments are in excellent agreement with those obtained from physiological response.

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