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Interaction of Tricyclic Antidepressants with the Ionic Channel of the Acetylcholine Receptor of *Torpedo* Electric Organ

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SUMMARY

Imipramine (IMIP) inhibited the binding of [^{3}H]perhydrohistrionicotoxin ([^{3}H]H_{12}-HTX) but did not inhibit the binding of [^{3}H]acetylcholine ([^{3}H]ACh) or [^{3}H]*d*-tubocurarine to *Torpedo* membranes. Binding of [^{3}H]IMIP to *Torpedo*, measured by a filter assay, was saturable, reversible, with high affinity (K_d 0.42 μM), and inhibited by channel blockers. In the presence of 20 μM carbamylcholine (carb) the affinity of [^{3}H]IMIP increased almost 4-fold, and the maximal number of binding sites and initial rate of binding increased 5-fold. Preincubation of *Torpedo* membranes with carb reduced this agonist-stimulated binding of [^{3}H]IMIP. [^{3}H]IMIP binding was stimulated by other agonists as well as by the antagonist *d*-tubocurarine, although to a much lower degree than agonists, and these increases were totally blocked by α-bungarotoxin. [^{3}H]IMIP binding to *Torpedo* membranes differed in two major aspects from [^{3}H]H_{12}-HTX binding. Its maximal binding in absence of carb was much lower than in its presence, and binding was little affected by changes in temperature, more like binding of receptor agonists which showed weak temperature dependency. It is suggested that [^{3}H]IMIP binds to sites on the ionic channel moiety of the receptor/channel molecules which are allosterically coupled to the ACh binding sites, but there are differences between binding of [^{3}H]IMIP and [^{3}H]H_{12}-HTX. The changes in [^{3}H]IMIP binding are suggested to be due to ligand-induced conformations of the ACh-receptor/channel molecule. Six tricyclic antidepressants (IMIP, nortriptyline, amitriptyline, desimipramine, protriptyline, and doxepin) blocked [^{3}H]H_{12}-HTX and [^{3}H]IMIP binding with slightly differing potencies, with doxepin as the least potent. It is suggested that this nicotinic ACh receptor/channel molecule is not a primary target for tricyclic antidepressant drugs or their cardiotoxicity, although similar ionic channels or carriers may be.

INTRODUCTION

TCA[2] drugs are currently the most widely used for treatment of depression (1). They have numerous pharmacologic effects. Reactions resulting from therapeutic doses of TCA drugs such as dry mouth, epigastric distress, constipation, dizziness, blurred vision, and urinary retention may be explained by their interaction with muscarinic cholinergic receptors (2). Similarly, effects on the cardiovascular system (e.g., lowering of blood pressure, tachycardia, or precipitation of congestive heart failure) may be explained by their inhibition of norepinephrine reuptake in sympathetic nerve terminals and the resultant high concentrations in cardiac tissue (3). However, the molecular mechanism of TCA action is still unknown, and many reactions are not easily explained by either mechanism, such as the rarely occurring weakness and fatigue associated with TCA therapy, the quinidine-like myocardial depressant effect, the excessive activation of sweat glands, and the transition from depression to manic excitement in some patients. Some of these may result from the interactions of TCA with ion transport processes of excitable membranes. IMIP has been found to block not only the effects of bethanechol, but also $BaCl_2$ on rabbit bladder (4), and its direct membrane action on the cardiac Purkinje fibers suggested that it interfered with Na^+ and other ionic conductances (5). On the voltage-clamped *Myxicola* squid giant axon, IMIP reversibly reduced both Na^+ and K^+ conductances without significantly changing the resting membrane potential or the leakage conductance (6). IMIP also inhibited the excitability and blocked impulse conduction of the desheathed frog sciatic nerves (7). On the single skeletal muscle fiber of the frog, IMIP had a dose-dependent

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[2] The abbreviations used are: TCA, tricyclic antidepressant; IMIP, imipramine; ACh, acetylcholine; H_{12}-HTX, perhydrohistrionicotoxin; carb, carbamylcholine.

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effect on the rate of rise and fall of the action potential (8, 9).

At neural and neuromuscular synapses these drugs have been shown to block the action of a variety of neurotransmitter receptors, such as muscarinic (10), histaminergic (11, 12), and α-adrenergic (13) receptors, as well as to inhibit uptake of biogenic amines (14). At motor end plates the anticholinergic action of TCA drugs was found to be noncompetitive (15, 16), possibly through their interactions with the ionic channel moiety of the ACh receptor (17, 18).

The nicotinic ACh receptor is an allosteric protein which traverses the membrane bilayer and extends beyond it 55 Å extracellularly and 15 Å intracellularly and has an ionic channel that probably extends through the whole molecule (19). It has sites that bind ACh in a voltage-independent manner and other sites whose binding of drugs is voltage-dependent and causes changes in the time course of end-plate current (20). It is not yet known where these latter sites are located, whether in the channel itself, in the intracellular or extracellular portions of the receptor molecule or possibly even at the interface between the receptor and the surrounding lipids. These are designated as "channel" or "allosteric" sites, while the ones that bind ACh and α-bungarotoxin are the "receptor" sites. In this study we report on the binding of [^{3}H]IMIP to the ACh receptor/channel molecule of the electric organ of the electric ray, *Torpedo* sp., the differences between [^{3}H]IMIP and [^{3}H]H_{12}-HTX binding to the ionic channel sites and the interactions of TCA drugs with these sites. An evaluation is made of these as targets for TCA action.

MATERIALS AND METHODS

Tissue preparation. Electric organs of *Torpedo ocellata* which had been stored at −90° were excised into ¼-inch cubes and homogenized in cold (4°) 10 mM Tris-HCl, pH 7.4 (1 g/ml), containing 1 mM EDTA 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM diisopropylfluorophosphate, and 0.02% NaN_3. The pellets of a 10-min centrifugation at 5,000 × *g* were suspended in the same buffer and recentrifuged under the same conditions. The supernatant fractions were combined and centrifuged at 30,000 × *g* for 60 min. The pellets were resuspended in 10 mM Tris-HCl (pH 7.4) and 0.02% NaN_3 at a protein concentration of 1–2 mg/ml. The number of ACh receptor sites ranged from 0.5–1 nmol/mg of protein.

Binding assays. Binding of [^{3}H]ACh (specific activity 90 mCi/mmole) and [^{3}H]*d*-tubocurarine (specific activity 20.6 Ci/mmole; New England Nuclear Corporation, Boston, Mass.) to *Torpedo* membranes was measured by equilibrium dialysis (21). A sample of the membrane preparation (250 μl) in tied dialysis tubing (5.6 mm, Union Carbide, pretreated to remove contaminants) was placed in 10 ml of Krebs original Ringer-phosphate solution (millimolar concentrations: NaCl, 107; KCl, 4.8; $CaCl_2$, 0.65; $MgSO_4$, 1.23; Na_2HPO_4, 15.7; pH 7.4). After 4 hr of shaking at 22°, three 50-μl aliquots were taken from bath and bag contents and counted in a liquid scintillation spectrometer. The excess radioactivity in tissue over bath sample represented the bound fraction. The amount of binding measured by this filter assay equaled that obtained by equilibrium dialysis. All detected binding to the *Torpedo* membranes was specific because it was totally inhibited with 10 μM α-bungarotoxin.

Binding of [^{3}H]H_{12}-HTX [specific activity 21 Ci/mmole obtained by tritiation of the natural alkaloid isodihydrohistrionicotoxin, and its activity tested on frog sartorius muscle (21)] and [^{3}H]IMIP (specific activity 29.8 Ci/mmole, from New England Nuclear) was measured by a filter assay (22). A membrane preparation (≃50 μg of protein) was incubated at 22° in 1 ml of 50 mM Tris-HCl, pH 7.4, containing the radiolabeled drug at 2 nM. The incubation mixture was vacuum-filtered on Whatman GF/B glass fiber filters (pretreated with 1% organosilane solution (Prosil-28, PCR Research Chemicals, Inc., Gainesville, Fla.) to reduce nonspecific binding, and the filters were washed with 10 ml of buffer, placed in 5 ml of scintillation fluid each, and their radioactive content was counted after at least 10 hr. Binding of [^{3}H]H_{12}-HTX and [^{3}H]IMIP was determined either in absence of (i.e., unstimulated binding) or presence of carb, an agonist which activates the receptor and opens the channel (i.e., stimulated binding). Specific binding was that blocked by 5 mM amantadine, a concentration which inhibited all specific binding (23). Nonspecific binding represented 50–60% of total unstimulated binding and 10–15% of total stimulated binding at saturation. When 100 μM nonradioactive IMIP was used to displace [^{3}H]IMIP specific binding, higher K_d values were obtained (17) than when 5 mM amantadine was used. This was discovered to be due to the competition of IMIP with the nonspecific [^{3}H]IMIP binding to the GF/B filters.

RESULTS

Effect of IMIP on binding of receptor and channel ligands. Whereas carb and gallamine at 10 μM were effective inhibitors of [^{3}H]ACh and [^{3}H]*d*-tubocurarine binding to *Torpedo* membranes, IMIP (at the same concentration) was not (Table 1). On the other hand, the same concentrations of IMIP inhibited all binding of 2 nM of the ionic channel probe [^{3}H]H_{12}-HTX as well as the carb-stimulated [^{3}H]H_{12}-HTX binding to *Torpedo* membranes (Fig. 1), suggesting that IMIP bound at a site other than the receptor site where carb bound. The IC_{50} value for IMIP inhibition of [^{3}H]H_{12}-HTX binding was calculated to be 250 nM.

Binding of [^{3}H]IMIP to Torpedo membranes. The specific binding of [^{3}H]IMIP (2 nM) to *Torpedo* membranes, measured after 120 min of incubation, was linear with the protein concentration up to 100 μg and was

TABLE 1

Effect of IMIP on the binding of [^{3}H]ACh (1 μM) and [^{3}H]d-tubocurarine (0.01 μM) to Torpedo electric organ membranes

Condition	Bound	
	[^{3}H]ACh	[^{3}H]*d*-tubocurarine
	pmoles/g tissue	
Control (no drugs)	874 ± 17	82 ± 2
Carb (10 μM)	160 ± 4	25 ± 1
Gallamine (10 μM)	544 ± 16	39 ± 2
IMIP (10 μM)	945 ± 42	82 ± 1

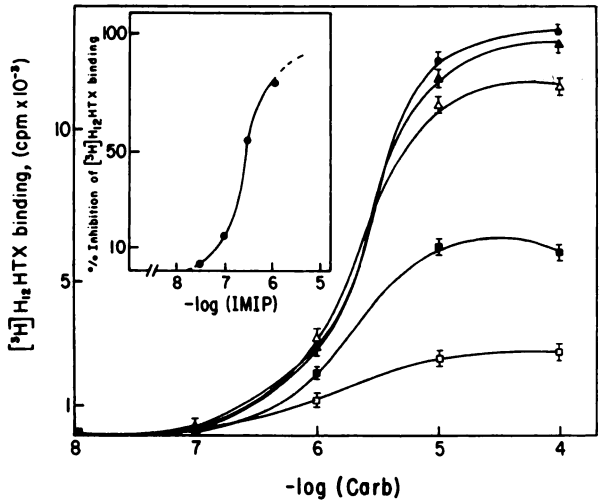

FIG. 1. *Effect of IMIP on the specific binding of [^{3}H]H_{12}-HTX (2 nM) (measured after 30 sec) to Torpedo membranes in presence of increasing concentrations of carb*

Control with no IMIP (●); IMIP, 30 nM (▲); IMIP, 100 nM (△); IMIP, 300 nM (■); and IMIP, 1 μM (□). Each *symbol* and *bar* represent the mean ± standard deviation of three experiments. The *inset* is a replot of the values of [^{3}H]H_{12}-HTX binding in presence of 100 μM carb and various IMIP concentrations.

enhanced by the presence of 20 μM carb (Fig. 2). When the time course of binding to *Torpedo* membranes was investigated it was found to reach equilibrium rapidly, such that binding at 1 min was not significantly different from that at 60 or 240 min (Fig. 3). Again, the presence of carb (20 μM) increased the extent of binding at this concentration ≃8-fold. When nonradioactive IMIP (100 μM) was added 30 min after incubation of [^{3}H]IMIP (2 nM) with *Torpedo* membranes there was an immediate displacement of bound [^{3}H]IMIP, indicating that this binding of IMP was rapidly reversible in presence of competing ligand (Fig. 3).

Saturation isotherms of the specific binding of [^{3}H] IMIP to *Torpedo* membranes in absence and presence of 20 μM carb were obtained by diluting [^{3}H]IMIP with nonradioactive IMIP (10^{-8}–10^{-5} M). Binding was to a single class of sites (Fig. 4A) which had K_d values of 0.42 μM and 0.11 μM in absence and presence of 20 μM carb, respectively, and a maximal number of sites of 49.7 ± 3.2

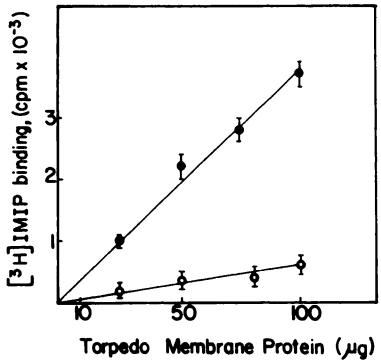

FIG. 2. *Binding of [^{3}H]IMIP (2 nM) to Torpedo membranes in absence (○) and presence (●) of 20 μM carb*

Incubation volume was 1 ml, and time was 120 min. Each *symbol* and *bar* represent the mean ± standard deviation of three experiments.

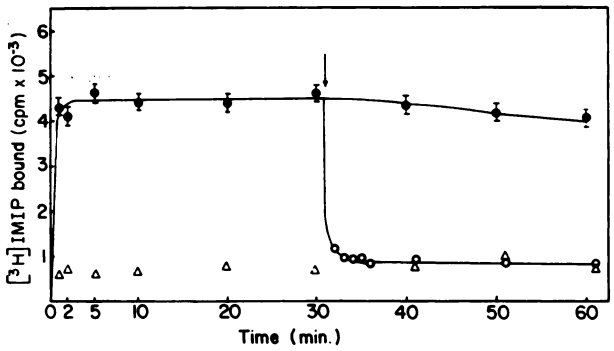

FIG. 3. *Kinetics of the specific binding of [^{3}H]IMIP (2 nM) to Torpedo membranes in absence (△) and presence (●) of 20 μM carb*

The *arrow* represents the time at which nonradioactive IMIP was added to 10 ml of the incubation mixture to give final concentration of 100 μM IMIP and displaced [^{3}H]IMIP binding (○). Each *symbol* represents the mean of three experiments and each *bar* represents the standard deviation.

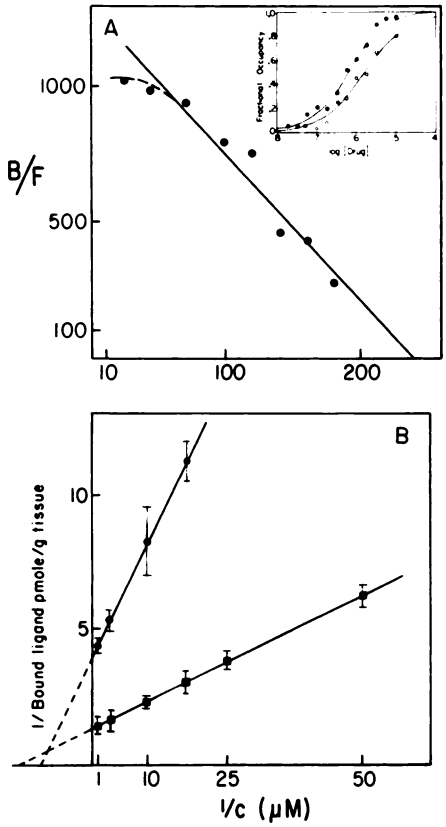

FIG. 4. *Specific binding of [^{3}H]IMIP to Torpedo membranes measured under equilibrium conditions (120-min incubation period, 1-ml incubation volume)*

A. Scatchard plot of the specific binding of [^{3}H]IMIP (diluted with nonradioactive IMIP) to *Torpedo* membranes in presence of 20 μM carb. *B* represents bound [^{3}H]IMP in picomoles per gram of tissue, and *F* represents free ligand concentration (nanomolar). The *inset* shows the binding (presented as fractional occupancy) as a function of IMP concentration in absence (○) and presence of 20 μM carb (●).

B. Double reciprocal plot of the binding of [^{3}H]IMIP (●) and [^{3}H] H_{12}-HTX (■) measured on the same tissue preparation. *C* is the concentration of free tritiated compound (micromolar). Each *symbol* and *bar* represent the mean ± standard deviation of three experiments.

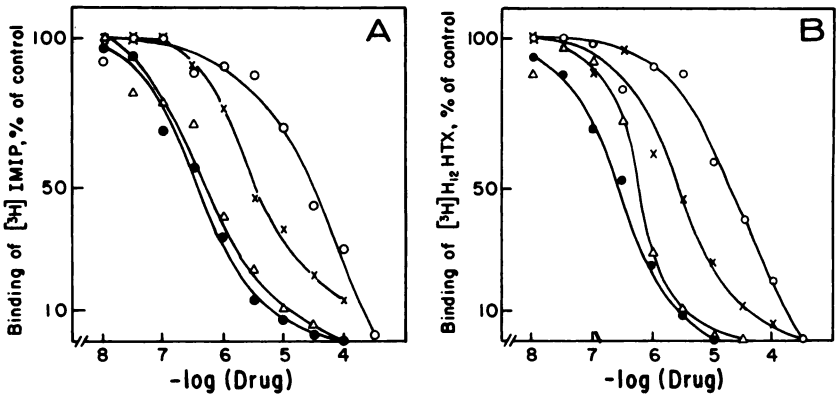

FIG. 5. *Effect of four inhibitors of the ionic channel of the ACh receptor on the binding of (A) [^{3}H]IMIP (2 nM) and (B) [^{3}H]H_{12}-HTX (2 nM) to Torpedo membranes in presence of 20 μM carb*

The value 100% represents the control obtained with the simultaneous addition of carb and [^{3}H]IMIP to the tissue. Phencyclidine (●), amantadine (○), quinacrine (△), and piperocaine (×). All incubation volumes were 1 ml, and times were 30 sec. Symbols represent average values of two experiments which showed less than 10% variation.

and 240 ± 10 pmoles/g of tissue, respectively. By comparison, binding of [^{3}H]H_{12}-HTX to the same membranes was to 750 pmoles/g of tissue and had a K_d of 80 nM (Fig. 4B).

Effect of channel drugs on the binding of [^{3}H]IMIP. Binding of [^{3}H]IMIP to *Torpedo* membranes was inhibited by the following drugs, which are known for their voltage-dependent action on the ACh receptor/channel at motor end plates and their inhibition of [^{3}H]H_{12}-HTX binding to *Torpedo* membranes: amantadine, quinacrine, piperocaine, and phencyclidine. The concentrations that inhibited 50% of binding (IC_{50}) of [^{3}H]H_{12}-HTX and [^{3}H]IMIP (Fig. 5) were similar.

Effect of receptor agonists and antagonists on the binding of [^{3}H]IMIP to Torpedo membranes. The effect of receptor agonists on the binding of [^{3}H]IMIP, after 30-sec exposure of tissue to carb and [^{3}H]IMIP simultaneously, was dose-dependent. The doses of ACh, carb, and decamethonium causing 50% of maximal stimulation of [^{3}H]IMIP binding were calculated from the log dose-response functions (Fig. 6) to be 0.2, 2, and 0.2 μM, respectively. At agonist concentrations higher than 100 μM there was a slight but significant decrease in [^{3}H]IMIP binding similar qualitatively to, although much less than, that observed with [^{3}H]H_{12}-HTX. In the case of decamethonium, higher concentrations caused a great reduction in the stimulation of [^{3}H]IMIP binding. Preincubation of *Torpedo* membranes with 20 μM carb for 1 min prior to introduction of [^{3}H]IMIP into the assay caused a rapid reduction in the stimulated binding (Fig. 7). However, there was only a very small reduction in [^{3}H]IMIP binding by preincubation with carb (Table 2). For example, after 1 min of preincubation of *Torpedo* membranes with 100 μM carb, the binding of [^{3}H]H_{12}-HTX was reduced by 58%, whereas the binding of [^{3}H]IMIP was reduced by only 16%.

A quaternary antagonist of the nicotinic receptor such as *d*-tubocurarine also increased the binding of [^{3}H]IMIP, but to a much lower degree (≃2-fold only) than agonists (Fig. 8). The stimulating effects of both agonist and antagonist on [^{3}H]IMIP binding were totally inhibited by prior treatment of the membranes with 10 μM α-bungarotoxin, suggesting that the stimulation of [^{3}H]

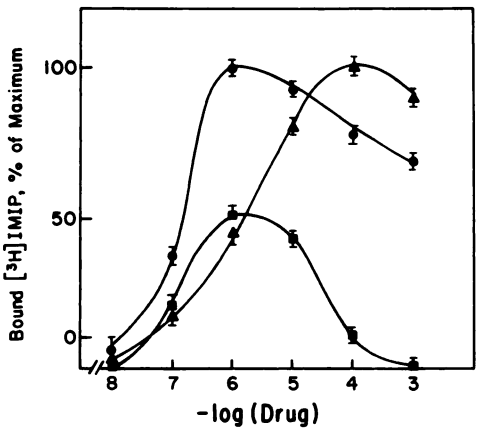

FIG. 6. *Effect of three receptor agonists on the specific binding of [^{3}H]IMIP (2 nM) to Torpedo membranes*

Acetylcholine (●), carb (▲), and decamethonium (■). Incubation time was 30 sec. Each *symbol* and *bar* represent the mean ± standard deviation of three experiments. The value 100% of binding is based on maximal binding obtained by ACh stimulation.

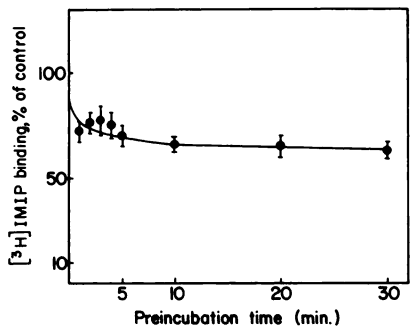

FIG. 7. *Effect of preincubation of Torpedo membranes with 20 μM carb for varied periods on the binding of [^{3}H]IMIP (2 nM)*

Carb-treated tissue was added to buffer containing [^{3}H]IMIP and carb, and incubated for 30 sec only. The value 100% control level was that obtained from the simultaneous addition of carb and [^{3}H]IMIP to the tissue. Each *symbol* and *bar* represent the mean ± standard deviation of three experiments.

TABLE 2

Effect of preincubation of Torpedo membranes with carb on their binding of [^{3}H]H_{12}-HTX and [^{3}H]IMIP

Channel probe (2 nM)	Preincubation time	Bound channel probe[a] in presence of carb (μM)		
		1	10	100
	min	*pmoles/g tissue*		
[^{3}H]H_{12}-HTX	0	5.1	12.2	13.8
	1	3.4	4.0	5.9
	30	4.2	4.8	5.0
[^{3}H]IMIP	0	1.6	3.8	3.3
	1	1.7	2.5	2.8
	30	1.9	2.5	2.4

[a] Standard deviations were <10%.

IMIP binding by both kinds of drugs was due to interactions with the receptor sites.

Effect of temperature on the binding of [^{3}H]IMIP. Our previous studies have indicated that the binding of [^{3}H]H_{12}-HTX was temperature-dependent, and Arrhenius plots showed positive correlation between temperature and affinity (20). The affinity of carb-stimulated [^{3}H]IMIP binding was determined from saturation isotherms obtained by displacing [^{3}H]IMIP by nonradioactive IMIP at 2, 15, 23, and 37°. The affinity constants at the four temperatures were calculated and plotted along with previously obtained values for [^{3}H]H_{12}-HTX, [^{3}H]ACh, and carb (Fig. 9). Whereas binding of receptor agonists was almost independent of temperature, that of the channel drug H_{12}-HTX was highly dependent. However, the carb-stimulated [^{3}H]IMIP binding was much less temperature-dependent than H_{12}-HTX in absence or presence of carb. When different concentrations of ACh were used to stimulate [^{3}H]H_{12}-HTX binding, there was an increase in both the affinity (represented by the apparent shift to the left of H_{12}-HTX binding) and extent of binding at 2° when compared with 22° or 37° (Fig. 10A). By contrast, the effect of temperature on the maximal response for ACh-stimulated [^{3}H]IMIP binding was reversed, with binding being highest at 2° and least at 22° and 37°, and there was no significant change in affinity (Fig. 10B).

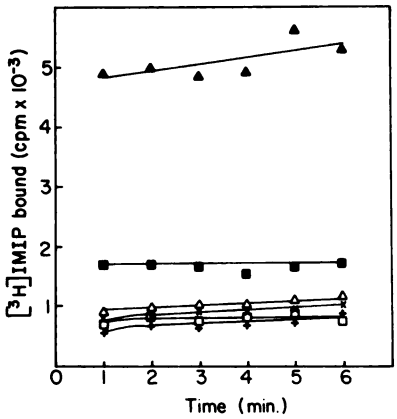

FIG. 8. *Binding of [^{3}H]IMIP (2 nM) to Torpedo membranes in absence (×) and presence of 20 μM carb (▲) or 20 μM d-tubocurarine (■), as well as binding to membranes pretreated for 1 hr with 10 μM α-bungarotoxin in absence (+) and presence of 20 μM carb (△) or 20 μM d-tubocurarine (□)*

Symbols are averages of two experiments with differences less than 10%.

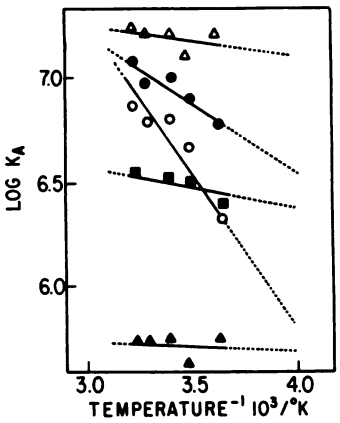

FIG. 9. *Arrhenius plot of the binding of [^{3}H]ACh (△), carb measured by displacing [^{3}H]ACh (▲), [^{3}H]H_{12}-HTX (○), [^{3}H]H_{12}-HTX in presence of 20 μM carb (●),and [^{3}H]IMIP in presence of carb (■)*

Effect of TCA drugs on binding of [^{3}H]H_{12}-HTX and [^{3}H]IMIP to Torpedo membranes. IMIP and five TCA drugs inhibited binding of [^{3}H]H_{12}-HTX to *Torpedo* membranes (Fig. 11), suggesting that all of them bound to the ionic channel moiety of the *Torpedo* ACh receptor. These drugs also inhibited binding of [^{3}H]IMIP with little differences in potencies and doxepin as the least potent on both radiolabeled probes (Fig. 12, Table 3).

DISCUSSION

Interactions of [^{3}H]IMIP with the nicotonic channel sites. Several findings suggest that [^{3}H]IMIP interacts with the nicotinic ACh receptor/channel molecule in *Torpedo* membranes through binding to the "channel" sites (as defined under Introduction). Binding is to a finite number of sites, is dependent upon protein concentration (Fig. 2), is with high affinity, and is inhibited by the following drugs which have been shown to interact with the ionic channel sites of the receptor/channel

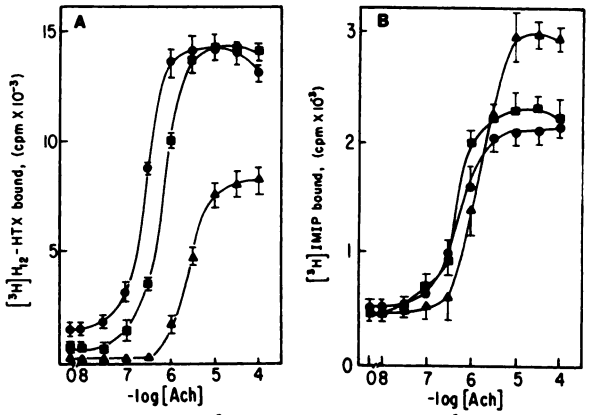

FIG. 10. *Binding of [^{3}H]H_{12}-HTX (A) and [^{3}H]IMIP (B) as a function of ACh concentration at three temperatures: 2° (▲); 22° (■), and 37° (●)*

Each *symbol* and *vertical bar* represent the mean ± standard deviation of three experiments.

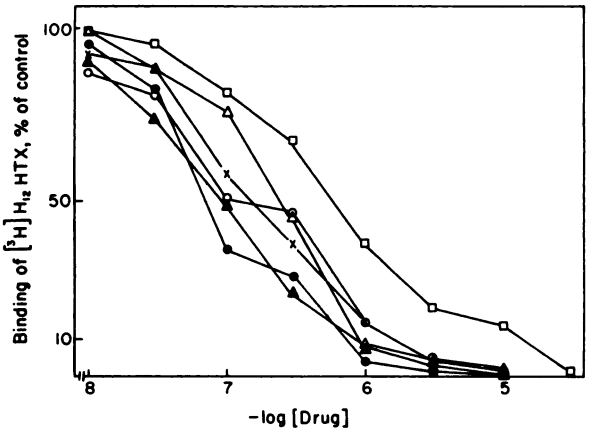

FIG. 11. *Effect of six TCA drugs on the binding of [^{3}H]H_{12}-HTX (2 nM) to Torpedo membranes*

The value 100% represents the control level of ^{3}H-labeled ligand binding in presence of 20 μM carb and absence of any other drugs. IMIP (○), desimipramine (×), nortriptyline (●), amitriptyline (△), protriptyline (▲), and doxepin (□). *Symbols* represent average values of two experiments which showed less than 10% variations. All incubation volumes were 1 ml, and times were 30 sec.

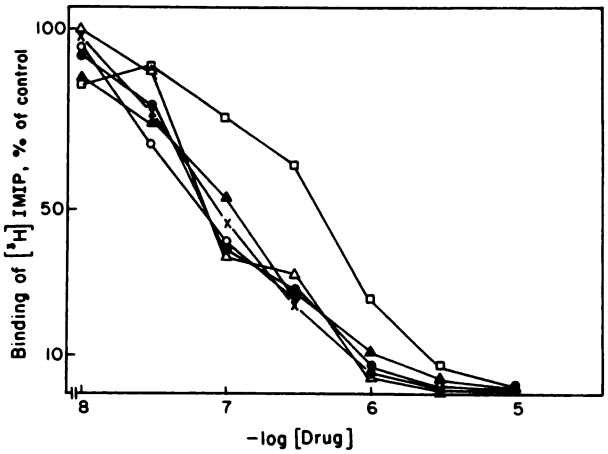

FIG. 12. *Effect of six TCA drugs on the binding of [^{3}H]IMIP (2 nM) to Torpedo membranes*

IMIP (○), desimipramine (×), nortriptyline (●), amitriptyline (△), protriptyline (▲), and doxepin (□). All other details as in Fig. 11.

molecule (Fig. 5): amantadine (23), quinacrine (24), piperocaine (26), and phencyclidine (27). In addition, IMIP has a voltage-dependent inhibitory effect on end-plate currents (17) and inhibits all [^{3}H]H_{12}-HTX binding (Fig. 1) at concentrations that do not inhibit [^{3}H]ACh, [^{3}H]*d*-tubocurarine (Table 1), or [^{3}H]α-bungarotoxin binding (17). It should be noted that, although *d*-tubocurarine interacts with both the ACh receptor sites and the channel sites of the receptor/channel molecule (28), over 99% of the binding of [^{3}H]*d*-tubocurarine at the concentration used (10 nM) is to the ACh receptor sites.[3]

It should be emphasized that the binding of [^{3}H]IMIP is determined after 30 sec, a period during which channels are activated as a result of agonist binding to the receptor which facilitates [^{3}H]IMIP binding, agonists and [^{3}H] IMIP dissociate, and even some receptors desensitize. These events occur repeatedly; thus, the amount of [^{3}H] IMIP bound reflects the summation of the binding to these conformations at 30 sec. The stimulation of [^{3}H] IMIP binding by receptor agonists (Fig. 6) demonstrates the allosteric effect of binding to the receptor sites on the channel sites and is evidence for the coupling between the two kinds of sites and IMIP binding's being to the channel sites. Although the action of [^{3}H]IMIP on the end plate is voltage-dependent (17), it is not known whether there is a potential difference across the *Torpedo* membranes used in the binding studies. If the potential is indeed near zero, then the stimulation of [^{3}H] IMIP binding presently observed is much smaller than that which would occur *in vivo*.

Comparison of [^{3}H]IMIP binding with [^{3}H]H_{12}-HTX. Binding of [^{3}H]IMIP to *Torpedo* membranes is similar in many respects to the binding of [^{3}H]H_{12}-HTX (17). Even at 100 μM, IMIP and H_{12}-HTX do not inhibit binding of [^{3}H]ACh or [^{125}I]α-bungarotoxin to the receptor sites, whereas their binding is inhibited by channel drugs (Fig. 5). The two drugs also cause nonlinearity in the end-plate voltage-current relationship (17, 21). The initial rates of their binding are increased by agonists (Fig. 6; ref. 22) with an order of potency similar to theirs in inducing muscle end-plate currents (29), and this stimulation is reduced by high agonist concentrations or preincubation with the agonist (Fig. 7; ref. 22), conditions that produce desensitized receptors. The antagonist *d*-tubocurarine, but not α-bungarotoxin, also increases the initial rates of binding of both [^{3}H]IMIP and [^{3}H]H_{12}-HTX, although to a much lower extent than agonists (Fig. 8; ref. 22). Since antagonists do not depolarize *Torpedo* membranes (30) and thus do not open channels, we have suggested that *d*-tubocurarine and other quaternary ammonium antagonists induce an inactive conformation of the ionic channel in which channel binding sites are more accessible in the presence of antagonists, in addition to the closed resting conformation (22). Thus, binding of [^{3}H]IMIP, like that of [^{3}H]H_{12}-HTX, is conformation-dependent.

However, there are differences betweeen IMIP and H_{12}-HTX binding to the ionic channel of the ACh receptor. Inhibition of [^{3}H]H_{12}-HTX binding to *Torpedo* membranes is noncompetitive (17), contrary to a recent report of competitive inhibition (18). Unlike [^{3}H]H_{12}-HTX and all other drugs that interact with this ionic channel, IMIP has only slight effect on the decay phase of the end-plate current, although it causes significant nonlinearity in the voltage-current relationship (17), like channel drugs do. Also, the total number of binding sites for [^{3}H]IMIP in the presence of carb is only one-third that for [^{3}H]H_{12}-HTX (Fig. 4B); the total number of sites for [^{3}H]IMIP at equilibrium is 5 times higher when the receptor is activated with carb (Fig. 4A). On the other hand, in the case of [^{3}H]H_{12}-HTX, the maximal number of binding sites is the same at equilibrium whether or not carb is present (22), although at 30 sec carb (22) or ACh (Fig. 10) increases binding greatly. The agonist effect is due to increased affinity for [^{3}H]H_{12}-HTX and accessibility of the binding sites. The apparent rates of association and dissociation of [^{3}H]IMIP (Fig. 3) to *Torpedo* membranes are much higher than those of [^{3}H]H_{12}-HTX (17), and

[3] M. Eldefrawi, unpublished results.

TABLE 3

Binding constants of six TCA drugs to various putative target proteins in the nervous system

Tricyclic antidepressant	Binding constants (nM)					
	Torpedo electroplax		Rat brain			
	[^{3}H]IMIP binding[a]	ob[^{3}H]H_{12}-HTX binding[a]	[^{3}H]IMIP binding[b]	Norepinephrine uptake[c]	Muscarinic receptor[d]	*Alpha*-adrenergic receptor[e]
Tertiary amines						
IMIP	110	140	3.4	20	78	58
Amitriptyline	123	260	12	20	10	24
Doxepin	830	630	144	—	44	—
Secondary amines						
Nortriptyline	116	70	—	11	57	71
Protriptyline	191	100	9.6	3	—	277
Desimipramine	143	160	8.6	1.5	170	148

[a] K_i values in inhibiting the binding of [^{3}H]IMIP (2 nM) and [^{3}H]H_{12}-HTX (2 nM) to *Torpedo* membranes in presence of 1 μM carb.

[b] Calculated from ref. 25.

[c] Concentrations that inhibited 50% of uptake into brain synaptosomes (14).

[d] K_i values obtained from inhibition of the muscarinic receptor antagonist [^{3}H]quinuclidinyl benzilate (10).

[e] K_i values obtained from inhibition of the ^{3}H-labeled *alpha*-adrenergic antagonist 2-(1′,6′-dimethoxy]-phenoxyethylamino)methylbenzodioxan (13).

preincubation with agonist causes a large reduction in stimulated [^{3}H]H_{12}-HTX binding, but a very small one in stimulated [^{3}H]IMIP binding (Table 2). Finally, temperature has a much stronger effect on [^{3}H]H_{12}-HTX than [^{3}H]IMIP binding (Fig. 10), such that the carb-stimulated [^{3}H]IMIP binding is very little affected by changes in temperature, almost like agonist binding (Fig. 9). This may suggest that H_{12}-HTX binding sites are located deeper in the receptor/channel molecule than are IMIP binding sites. These actions of IMIP led to the suggestion that it binds preferentially to the activated but nonconducting conformation of the receptor/channel molecule (17).

Interactions of TCA with the nicotinic channel and relevance to their antidepressant and toxic actions. The five TCA drugs tested, like IMIP, bind to the ionic channel of the receptor/channel molecule, since they inhibit the specific binding of [^{3}H]H_{12}-HTX (Fig. 11) and [^{3}H]IMIP (Fig. 12) to these channel sites, although with slightly differing potencies (Table 3). TCA drugs bind with high affinities to several macromolecules that are found in rat brain (Table 3). The recommended upper limits for daily doses of TCA drugs in treating depression are 150 mg for amitriptyline and 200 mg for IMIP (11), which yield plasma concentrations of 0.3–0.8 μM. The affinities of TCA drugs to the ACh receptor/channel sites [K_i 0.11–0.83 μM (Table 3)] fall within these therapeutic concentrations, thus suggesting that the peripheral nicotinic ACh receptor/channel may be a secondary target for TCA drugs. However, it is very difficult to correlate the affinity of TCA drugs for the ACh receptor/channel sites with their antidepressant action, the reasons being the varied order of potency of TCA drugs against different types of depression. We found no correlation between the affinity of TCA drugs for this peripheral nicotinic ionic channel and the therapeutic success index value, which is a ratio of percentage success in treating patients with agitated depression by percentage success in treating patients with retarded depression (13).

There is no distinction between secondary and tertiary amine TCA drugs in their effects on the nicotinic ionic channel (Table 3). On the other hand, secondary amine TCA drugs are more potent than the tertiary amines in inhibiting norepinephrine uptake (13), [^{3}H]IMIP binding to rat brain synaptosomes (25), and in causing psychomotor activation (13), but are less potent in inhibiting binding to *alpha*-adrenergic receptor and in decreasing blood pressure and heart rate (13). The finding that doxepin is the least potent of the TCA drugs tested on the peripheral nicotinic ionic channel (Figs. 11 and 12), yet the most potent in producing sedation and hypotension (13), argues against the involvement of a similar ionic channel in these actions. Nicotonic ACh receptors are found in the central nervous system (31), but their channel properties are not known.

Doxepin is much less potent than other TCA drugs in causing quinidine-like delays in intracardiac conduction, which makes it the preferred TCA drug for use in cardiac patients (32). If cardiotoxicity of TCA drugs is due to an action on an ionic channel in the heart, then this channel may share some drug specificity with the ionic channel of the nicotinic ACh receptor. It should be noted that there are no postsynaptic nicotinic receptors in heart.

Another possibility is that binding of TCA drugs to the *Torpedo* nicotinic channels may correlate with inhibition of biogenic amine uptake. Amantadine, which inhibits the uptake of dopamine and is used as an antiparkinson drug, is an inhibitor of these channels and binding of [^{3}H]H_{12}-HTX to *Torpedo* membranes (23). Similarly, phencyclidine, which is also known to inhibit the uptake of norepinephrine and 5-hydroxytryptamine into rat brain synaptosomes (33), inhibits this ionic channel and binding of [^{3}H]H_{12}-HTX to *Torpedo* membranes (27). Both drugs displace [^{3}H]IMIP and [^{3}H]H_{12}-HTX binding with similar affinities (Fig. 5).

In conclusion, it is clear that IMIP and other TCA drugs bind to, and inhibit, the ionic channel of the nicotinic ACh receptor, but this molecule is unlikely to be the primary target for their pharmacological or cardiotoxic effects.

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