cubated intact MCF-7 cells with physiological ($10^{-8}M$) and pharmacological ($10^{-6}M$) concentrations of dihydrotestosterone (DHT) and then examined the effects on receptors for estrogen, androgen, progesterone, and glucocorticoids. With $10^{-8}M$ concentrations of DHT, cytoplasmic androgen receptor was translocated to the nucleus while the other receptors remained unaltered. The higher dose of DHT, however, depleted not only the androgen receptor but also a significant portion of the estrogen receptor; neither progesterone nor glucocorticoid receptor levels were affected. Figure 1 shows that after the cytoplasmic depletion of estrogen receptor with $10^{-6}M$ DHT a near equal number of estrogen receptors entered the nucleus.

The cytoplasmic depletion and translocation of the MCF-7 estrogen receptor was not limited to DHT; other androgens such as testosterone and androstanediol were equally effective, whereas high concentrations or progesterone or hydrocortisone did not bind this receptor. Thus, androgens appear to have a specific low affinity for the estrogen receptor.

If the DHT-translocated nuclear estrogen receptor is functional, then specific products of estrogen action should be induced. Progesterone receptor is such a product of estrogen action. Low and high concentrations of DHT were therefore compared for their ability to induce progesterone receptor. When intact cells were exposed to $10^{-8}M$ DHT, progesterone receptor levels remained unaltered, whereas in cells exposed to $10^{-6}M$ DHT progesterone receptors were significantly stimulated (Fig. 2). Thus the androgen-translocated nuclear estrogen receptor must be active at specific gene acceptor sites, inducing products normally considered to be restricted to the action of estrogen.

For these data to be clinically relevant, it must be shown that pharmacological concentrations of androgens are capable of stimulating breast tumor growth in vivo under certain conditions. Heise and Gorlich (*5*) studied the effect of various doses of androgen on breast tumor regression in rats treated with dimethylbenzanthracene. They found that very high concentrations of androgen were much less effective in causing tumor regression than lower concentrations of androgen; in some instances, the very high concentrations of androgen even appeared to stimulate tumor growth. Similar results were reported by Segaloff (*6*).

Although our data provide evidence that $10^{-6}M$ DHT stimulates MCF-7 cell growth by acting through the estrogen receptor, and that this may also account for the breast tumor stimulation in vivo in the rat, they do not provide direct evidence that these events occur in a clinical setting in human breast cancer. Nevertheless, the possibility remains that the failure of androgen to induce tumor regression in certain breast cancer patients may be related to the paradoxical estrogenic effects of pharmacological concentrations of androgens.

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Amantadine: Neuromuscular Blockade by Suppression of Ionic Conductance of the Acetylcholine Receptor

Abstract. *Amantadine hydrochloride decreases the sensitivity of denervated mammalian muscle to iontophoretically applied acetylcholine. The drug depresses the amplitude of the end-plate current and reverses the slope of the relation between half-decay time and membrane potential suggesting that it alters the ionic conductance that is mediated by the acetylcholine receptor. Binding studies confirm that amantadine acts on the ion conductance modulator rather than the acetylcholine receptor.*

At neuromuscular junctions, the acetylcholine (ACh) receptor serves as the recognition site for ACh released from motoneurons. Binding of ACh to its receptor induces permeability changes in the postjunctional membrane through activation of the ion conductance modulator (ICM). Our recent studies suggest that the ACh receptor and ICM are separate membrane proteins that are coupled in the membrane (*1*). This ICM may consist of a channel and structures that link the channel to the receptor. The ionic conductances that are mediated at neuromuscular junctions by the ACh receptor may be blocked by drugs or toxins that act directly on the receptor (for example, the receptor antagonists *d*-tubocurarine and α-bungarotoxin) or on the ICM (for example, local anesthetics and histrionicotoxin) (*1*-*3*).

Amantadine hydrochloride (1-adamantanamine hydrochloride, Symmetrel) is an antiviral drug known for its prophylactic effect against A_2 (Asian) influenza in animals and man (*4*). Its antiviral activity is suggested to result from the ability of the drug to prevent viral penetration into the host cell (*5*). This drug is also effective in the treatment of human Parkinsonism (*6*). Though it is considered likely that amantadine exerts its anti-Parkinson effect through an action similar to that of L-dopa (*6*), the drug has recently been shown to block neuromuscular transmission by reducing the response of muscle postjunctional membrane to ACh (*7*). Since such an effect may result from actions on the ACh receptor or its ICM, we utilized biochemical and electrophysiological techniques to distinguish between these possibilities and to elucidate the molecular target of amantadine.

For the biochemical study, membranes were prepared from a tissue extremely rich in nicotinic neuromuscular-type ACh receptors and ICM's, namely, the electric organ of the electric ray, *Torpedo ocellata* (*8*). The ACh receptor was identified by its binding of [^{3}H]ACh (49.5 Ci/mole, New England Nuclear) after inhibition of all the acetylcholinesterase present with $10^{-4}M$ diisopropylfluorophosphate. The ICM was identified by its binding of [^{3}H]perhydrohistrionicotoxin ([^{3}H]H_{12}-HTX; 4800 Ci/mole). The effect of this radioactively labeled toxin on end-plate current (EPC) was checked, and its binding (dissociation constant, $K_d = 4 \times 10^{-7}M$) was saturable, specific, and blocked only by drugs and toxins shown to affect the ionic conductance of the end plate (*1*). A membrane preparation was made from the electric organ (600 pmole of receptor sites per milligram of protein), and binding was studied by equilibrium dialysis at 22°C for 4 hours as previously described (*1*).

Amantadine significantly reduced the

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binding of [^{3}H]H_{12}-HTX at concentrations that did not affect [^{3}H]ACh binding (Table 1). Another ligand that gave similar results was the histrionicotoxin octahydro isomer (H_8-HTX). On the other hand, [^{3}H]ACh binding was abolished by prior incubation of the membranes with 1 μM α-bungarotoxin and was blocked to varying degrees by carbamylcholine and *d*-tubocurarine. These receptor ligands had little or no effect on the binding of [^{3}H]H_{12}-HTX (Table 1). These results suggested that the neuromuscular blocking action of amantadine might be due to a histrionicotoxin-like effect resulting from binding of amantadine to the ICM rather than to the ACh receptor.

The effect of amantadine on the ACh sensitivity of muscle fibers was studied on denervated (14 to 17 days) soleus muscles of the rat (*9*). When the preparation was treated with $1 \times 10^{-4} M$ amantadine for 30 minutes, a marked reduction in the sensitivity to microiontophoretically applied ACh was observed. The extrajunctional ACh sensitivity was reduced from 690 ± 78 mV/nC (N = 6) to 183 ± 43 mV/nC (N = 3). Higher concentrations of amantadine caused further reductions in ACh sensitivity. These results are in agreement with the effect of amantadine on ACh sensitivity of frog muscles reported by Nastuk *et al.* (*7*). However, such a reduction in sensitivity could result from effects on the receptor as well as the ICM. In order to distinguish further the site or sites of action of amantadine, we used a modified voltage-clamp technique to record the EPC of the surface fibers of frog sartorius muscle (*10*). The muscles were first treated with hypertonic glycerol Ringer solution (500 to 600 mM), and then returned to normal Ringer solution to eliminate contraction during membrane depolarization (*11*).

It is well known that drugs which act on the ACh receptor site, such as *d*-tubocurarine, depress the peak amplitude of the EPC, but do not appreciably alter its time course, nor the dependence of peak amplitude and half-decay time on membrane potential (see Fig. 1). In contrast, agents which act on the ICM, such as some local anesthetics and the histrionicotoxins (*1*), alter both the EPC amplitude and time course and modify the influence of membrane potential on the peak amplitude and half-decay time. The effects of amantadine ($2 \times 10^{-4} M$) on the EPC at −90 mV are presented in Table 2. Under control conditions, the EPC had a rise time of 0.80 msec, a peak amplitude of 0.402 μA, and a half-decay time of 1.60 msec. Thirty minutes after the addition of amantadine, the EPC amplitude was depressed by 70 percent ($P \leq .01$), and the rise and half-decay times were shortened by 13 and 31 percent, respectively ($P \leq .001$). These drug-induced alterations of the EPC time course were reversed by washing the preparations for 60 to 120 minutes with drug-free solution.

Figure 1A shows that the peak amplitude of the control EPC responded linearly to changes in the driving force at potentials between +60 and −100 mV, and exhibited a small curvature at potentials between −100 and −160 mV (*12*). In the presence of amantadine ($2 \times 10^{-4} M$), the linear segment was curtailed, extending only from +60 to −10 mV. Beyond −10 mV, the current voltage relation became markedly nonlinear and showed a characteristic region of negative conductance at potentials greater than −120 mV. These alterations resemble those

Table 1. Blockade of binding of [^{3}H]acetylcholine and [^{3}H]perhydrohistrionicotoxin to ACh receptor enriched membranes from the electric organ of *T. ocellata* measured by equilibrium dialysis.

Drug or toxin	[^{3}H]Perhydrohistrionicotoxin (40 nM)		[^{3}H]Acetylcholine (1 μM)	
	Concentration* (μM)	Binding as percentage of control	Concentration (μM)	Binding as percentage of control
Carbamylcholine	100	86 ± 8	10	10 ± 3
d-Tubocurarine	100	103 ± 4	10	41 ± 5
α-Bungarotoxin	1	126 ± 7	1	1 ± 2
H_8-HTX	1	38 ± 2	1	99 ± 6
Amantadine	10	72 ± 2	10	108 ± 7
Amantadine	100	46 ± 1	100	100 ± 2

*The drug or toxin was added to the dialysis bath at the final indicated concentration except for α-bungarotoxin, which was added to both the dialysis bag contents as well as to the bath.

Table 2. Effect of amantadine ($2 \times 10^{-4} M$) on the amplitude and time course of end-plate current recorded at −90 mV. N shows the number of fibers tested. The preparations were washed for 60 to 120 minutes.

Condition	Amplitude (nA)	N	Rise time (msec)	Half-decay time (msec)
Control	402 ± 46	23	0.80 ± 0.02	1.60 ± 0.03
Amantadine	120 ± 19*	25	0.70 ± 0.03†	1.10 ± 0.06†
After washing	353 ± 43	8	0.80 ± 0.03	1.59 ± 0.03

*Difference from control value is statistically significant ($P < .01$). †Difference from control value is statistically significant ($P < .001$).

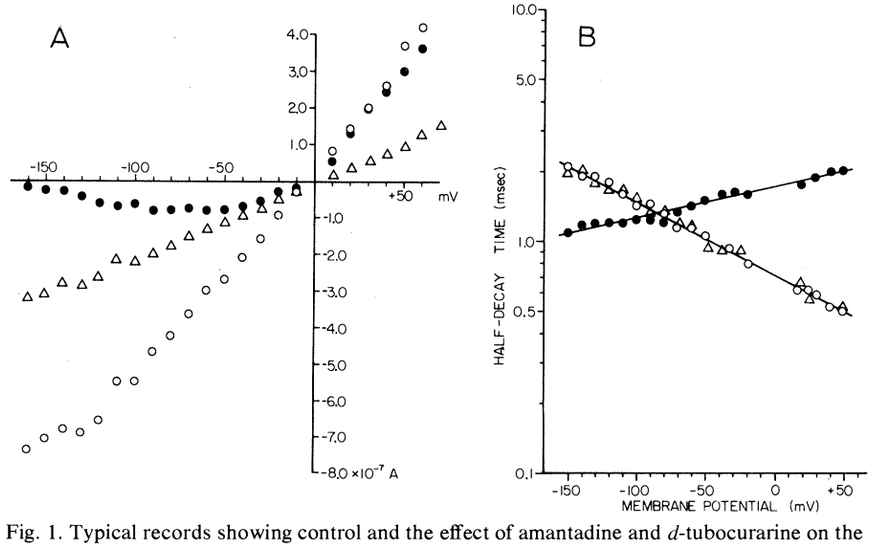

Fig. 1. Typical records showing control and the effect of amantadine and *d*-tubocurarine on the end-plate currents of the surface fibers of sartorius muscles of frog. (A) Relation between amplitude of EPC (inward current, negative; outward current, positive) and membrane potential. Control (○); in the presence of amantadine at $2 \times 10^{-4} M$ (●) or *d*-tubocurarine at $2 \times 10^{-6} M$ (△). (B) The relation between the half-decay time of the EPC and the membrane potential in normal Ringer solution (○) and during exposure to amantadine at $2 \times 10^{-4} M$ (●) or *d*-tubocurarine at $2 \times 10^{-6} M$ (△).

produced by histrionicotoxin (*1*), and suggest that amantadine's action is voltage-dependent.

This conclusion receives additional support from observations that amantadine alters the relation between the EPC half-decay time and membrane potential. The half-decay time of the control EPC varied exponentially and became shorter as the membrane potential was driven from −150 to +60 mV (Fig. 1). In the presence of amantadine ($2 \times 10^{-4} M$) the slope of the relation between half-decay time and membrane potential underwent a very striking reversal such that the EPC's now became faster with hyperpolarization. Although small shifts in the slope for half-decay time versus membrane potential have been reported before (*13*) they have never been as complete as with amantadine. Thus, during exposure to amantadine the slope of the logarithm of the half-decay time was $+1.344\ V^{-1}$ and under control condition was $-3.05\ V^{-1}$. Consequently, amantadine appears to be a useful pharmacological probe for examining the coupling of the end-plate channel kinetics with the membrane electrical field.

These findings raise several questions relating to the other effects of amantadine. How does amantadine block virus entry into host cells? Is amantadine selective for the peripheral nicotinic cholinergic ICM or does it also affect central cholinergic ICM or possibly ICM's associated with other receptors? Does the beneficial effect of amantadine in Parkinsonism depend on the capacity of this drug to inhibit ionic conductance in certain neuronal circuits involved in the central control of muscular movement? Answers to these and similar questions will undoubtedly improve our understanding of basic biological mechanisms and the mode of action of the drug.

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"Wolf-in-Sheep's-Clothing" Strategy of a Predaceous Insect Larva

Abstract. *The larva of the green lacewing* Chrysopa slossonae *lives in colonies of the wooly alder aphid* Prociphilus tesselatus *upon which it feeds. It disguises itself as its prey by plucking some of the waxy "wool" from the bodies of the aphids and applying this material to its own back. The investiture protects it from assault by the ants that ordinarily "shepherd" the aphids. Larvae artifically denuded are seized by the ants and removed from the aphid colonies. A larva requires on the average less than 20 minutes to coat itself with wax. A hungry denuded larva gives the coating procedure about the same behavioral priority as feeding.*

Aphids, except when dispersing by flight, are relatively immobile and need little carbohydrate. But they are extraordinarily prolific and hence require nitrogenous materials in disproportionately large amounts. They feed by pumping large quantities of plant juices through their bodies, thereby meeting the nitrogenous demands but also inevitably imbibing excess carbohydrate. They void this excess as part of their excreta, a sweet fluid appropriately called honeydew. As is well known, aphids do not necessarily waste this honeydew, but may present it as an offering to ants, which drink the fluid and in exchange shepherd the aphids and provide them with protection. Predators intent on feeding on aphids must contend with such ants, which are provenly aggressive when guarding their aphid flock (*1*). We now describe the extraordinary behavior of a predaceous insect larva that feeds on aphids and copes with ants by masquerading as an aphid.

The larva, a member of the aphidophagous family Chrysopidae (order Neuroptera), lives in colonies of the wooly alder aphid *Prociphilus tesselatus*. Previously undescribed as a larva (*2*), it was identified as *Chrysopa slossonae* after being raised to adulthood. Its association with *P. tesselatus* appears to be obligatory; we found it with no other aphids. At the various sites in Tompkins and Albany counties, New York, where we made our observations, it is relatively plentiful. From August to October, hardly a colony of *P. tesselatus* can be found that does not harbor at least several of these chrysopid larvae.

Prociphilus tesselatus derives its wooly appearance from the fluffy investiture of brilliantly white wax that covers its body (Fig. 1B). This material, recently identified as a long-chain ketoester (*3*), is secreted in the form of dense tufts of thin filaments from patches of integumental glandular cells. It renders the aphids extremely conspicuous against the dark branches of the alder bushes (*Alnus rugosa*) on which they typically occur (Fig. 1A). Three species of ants, all of the subfamily Formicinae, were

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