least three times the expected values. In the B and A regions of the genes, actual values were much closer to expected values. Pseudorandom sequences maintaining the dinucleotide frequencies of each region were also generated. Calculation of μ and σ and expectation values were made as above (see legend to Fig. 3) and led to the same conclusions as those obtained with regional chains generated with single nucleotide frequencies maintained.

In addition, pseudorandom chains of the same base composition as the entire genes were generated and also compared with appropriated consensus sequences (see legend to Fig. 3). If a Gaussian distribution is assumed, E.D. values $\leqq (\mu - 2\sigma)$ have a maximum probability of occurrence of $\sim .023$ (*7*).

The relation between the Sp and the latter part of the C region is therefore significant and not a fortuitous result of similar base composition or dinucleotide frequency. For the chicken, no significant regions of E.D $\leqq (\mu - 2\sigma)$ calculated for all three types of pseudorandom chains were found outside of the Sp region from which the consensus sequence was derived.

We speculate that the pre-proinsulin gene evolved from two separate minigenes, each with identical or nearly identical Sp regions, but separate functional regions corresponding to the B and A regions of the modern gene. Primitive pre-proinsulin may have arisen through selection pressures favoring the juxtaposition of these two minigene products so that proper disulfide bridges could form more easily in the maturation of the hormone. The functional genomic units, mediated by the large intron, evolved into a single transcriptional unit. The nucleotide sequence coding for the Sp region of the putative A minigene became the latter portion of the C region. Evolution into a functional C peptide coding region probably occurred at a very rapid rate because of the great advantage of such a unit for proper folding and disulfide bridge formation. This model is depicted in Fig. 4.

The evolutionary relationship between the Sp regions of the four pre-proinsulins examined in this study implies a common origin. In the context of the foregoing speculation, at least the signal peptide coding portion of the putative minigenes must have arisen from a common ancestral source, probably by gene duplication.

An alternative interpretation of our findings is that an ancient pre-proinsulin precursor was made up entirely of tandem repeats. Divergent evolution with strong selection pressures for particular amino acids in the functional B and A regions could have obliterated any identifiable remnant of the primordial base repeats, or for that matter, of any other homologous relation. This alternative model does not account for the existence and possible evolutionary role of the large intron.

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Neurotoxin-Specific Immunoglobulins Accelerate Dissociation of the Neurotoxin–Acetylcholine Receptor Complex

Abstract. *Toxin isolated from cobra venom and labeled with tritium was incubated with membranes rich in acetylcholine receptors. The amount of toxin bound to the receptors was determined and the kinetics of dissociation of the receptor-toxin complex was followed. Addition of an excess of horse antiserum to the venom resulted in a significant acceleration of the dissociation reaction. Similarly, a monoclonal antibody against the toxin accelerated dissociation of the receptor-toxin complex. The results indicate that specific antibody binding destabilizes the toxin-receptor complex.*

Injection of cobra venom into mammals produces a flaccid muscle paralysis, and death occurs by respiratory deficiency (*1*). The lethal effect is due to the presence in the venom of neurotoxic proteins (molecular weight, 6000 to 7000), which bind tightly and specifically to the nicotinic acetylcholine (ACh) receptor, producing a nondepolarizing block (*2*). Therapeutic action against cobra bite usually consists of treatment with specific antibody to the venom (*3, 4*). This action is commonly explained by the fact that one free neurotoxin molecule binds to at most three or four specific immunoglobulin molecules and thus becomes inactive (*5, 6*).

However, recent data suggest that neurotoxin-specific immunoglobulins may have a role at the level of the neurotoxin–ACh receptor complex. It is known that (i) a neurotoxin molecule associated with its physiological target is not internalized but remains at the surface of the postsynaptic membrane (*7, 8*) and that (ii) the binding of a neurotoxin to its

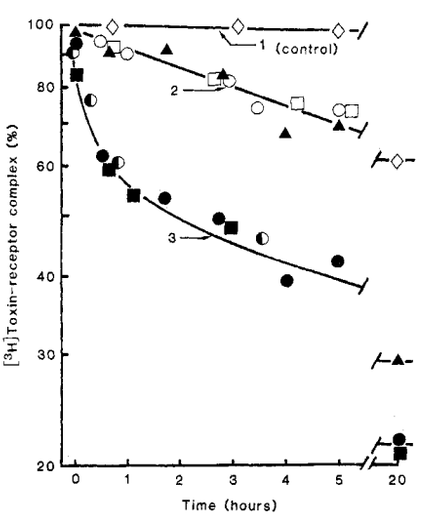

Fig. 1. Effect of excess unlabeled toxin or antibodies to the toxin on dissociation of the [^{3}H]toxin-receptor complex. Acetylcholine receptor–rich membranes from *T. marmorata* (10 n*M* [^{3}H]toxin binding sites) were mixed with [^{3}H]toxin (4 n*M*) in Ringer solution at room temperature. After incubation for 3 hours or overnight the mixture was diluted ten times. Reversal was initiated by adding (▲) unlabeled toxin at a final concentration of 0.7 μM, (■) horse antiserum to the venom (0.3 μM), (●) mouse monoclonal antibody against the toxin, or (◐) its Fab fragments at a final concentration of 0.25 μM; or a preincubated (30 minutes) mixture of toxin (0.7 μM) and either (□) horse antiserum (0.3 μM) or (○) monoclonal antibody (0.25 μM). When the toxin-antibody complex is formed, the amount of free unlabeled toxin remains large (at least 1000 times the concentration of [^{3}H]toxin). The control (◇) was prepared without adding unlabeled toxin or antitoxin immunoglobulins. At different times 0.5 ml of each mixture was taken and filtered through two Millipore filters (0.45 μm), which were washed with 15 ml of Ringer solution at 4°C. The filters were dried and the retained tritium was counted.

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receptor is centered on 12 residues which are mostly located on the same side of the neurotoxin molecule, leaving a large part of the toxin accessible from outside the membrane (*9*). Therefore, ACh receptor–bound toxin may be a target for one or more neurotoxin immunoglobulins. To determine whether such interactions between bound toxin and specific antibodies occur and, if they do, whether they affect the extremely stable neurotoxin-receptor complex, we studied the effect of horse antiserum to *Naja nigricollis* venom and of monoclonal neurotoxin–specific immunoglobulin on the dissociation of the neurotoxin–ACh receptor complex in vitro. We found that the dissociation of the complex was significantly accelerated by the neurotoxin-specific immunoglobulins we studied.

Toxin α isolated from *N. nigricollis* venom (*10*) and heavily labeled with tritium (*11*) was incubated with ACh receptor–rich membranes isolated from *Torpedo marmorata* (*12*) until equilibrium was reached. The amount of ^{3}H-labeled toxin bound to ACh receptor was determined by a filtration assay and the kinetics of dissociation was followed under a variety of conditions. To observe the dissociation of the [^{3}H]toxin-receptor complex it is necessary to prevent reassociation of [^{3}H]toxin molecules on the receptor—for instance, by trapping the free receptor or [^{3}H]toxin molecules or by diluting the solution. The results obtained are shown in Fig. 1. Addition of an excess of nonradioactive toxin saturates free receptor molecules and thus a slow decrease of bound [^{3}H]toxin is observed (curve 2 in Fig. 1). A great dilution (1:100) of the solution produces similar results (data not shown), indicating that there is no cooperative effect involving toxin molecules during the dissociation process. Addition of an excess of horse antiserum to *N. nigricollis* venom results in a significant acceleration of the dissociation, with 40 percent compared to 5 percent dissociated after 1/2 hour (curves 3 and 2, respectively, in Fig. 1). This effect of immunoglobulins against venom components is specific, since it does not occur when a normal horse serum is added (data not shown). Moreover, prior incubation of the specific serum with a large excess of nonradioactive neurotoxin prevents the accelerating effect (curve 2), indicating that the immunoglobulins responsible for this phenomenon are neurotoxin-specific. This effect cannot be explained by simple trapping of free [^{3}H]toxin molecules because, in that case, one would expect to observe dissociation kinetics similar to curve 2. Therefore, there may be an additional process which involves a direct effect of immunoglobulins on [^{3}H]toxin molecules bound to the receptor, inducing destabilization of the complex.

Recently, a monoclonal antibody against *N. nigricollis* toxin α was produced (*13*). The antigenic site at which this immunoglobulin binds has been identified and is distinct from the ACh receptor binding site of the toxin. As shown in Fig. 1, addition of this homogeneous toxin-specific immunoglobulin population accelerates the dissociation in a manner similar to that observed with the antiserum to the venom. This effect is inhibited when the antibody combining site is initially blocked by unlabeled toxin, confirming the importance of specific recognition of the antibody for the toxin molecule. The same effect is observed with Fab fragments (*14*) (Fig. 1).

The results presented here show that the neurotoxin–ACh receptor complex is appreciably destabilized in vitro by neurotoxin-specific immunoglobulins. This effect probably results from a direct interaction between at least one accessible antigenic determinant of the ACh receptor–bound toxin molecule and the corresponding antibody combining site. Whether the effect is dependent on the induction of a conformational change in the bound toxin remains to be determined. If the destabilizing effect is confirmed in vivo, it may be important for establishing an adequate serotherapy.

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Psoralen Phototoxicity: Correlation with Serum and Epidermal 8-Methoxypsoralen and 5-Methoxypsoralen in the Guinea Pig

Abstract. *Serum and epidermal concentrations of 8-methoxypsoralen and 5-methoxypsoralen 2 hours after oral administration to guinea pigs were determined by high-performance liquid chromatography. A linear relation was found between the serum and epidermal concentrations of 8-methoxypsoralen. In addition, a relation was found between serum concentrations of 5-methoxypsoralen and 8-methoxypsoralen and the appearance of phototoxicity. The lower phototoxicity of orally administered 5-methoxypsoralen as compared to 8-methoxypsoralen in the guinea pig appears to be due to its reduced concentrations in the epidermis, the primary site of the phototoxic events.*

Psoralens are a class of compounds present in many plants. These naturally occurring psoralens, such as 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP), and unsubstituted psoralen (Fig. 1), have been identified as phytoalexins; they are part of the plant's complex defensive response against fungal and insect (*1*) challenges. Furthermore, psoralens are powerful phototoxic agents in animals and humans. Humans can be exposed topically by contact with psoralen-containing fruits and vegetables (*2*) or cosmetics (*3*) and orally by ingestion of certain common fruits and vegetables (*4*) or the use of psoralen-containing drug formulations (*5*).

Psoralens are increasingly used in photochemotherapy for management of such disorders as vitiligo, psoriasis, and mycosis fungoids (*6*). This application involves oral administration of the chosen psoralen, typically 8-MOP, followed by irradiation of the patient with ultraviolet A (UVA) light (*7*). A well-designed photochemotherapy regimen, which produces the maximum therapeutic effect while eliciting the minimum phototoxic response, requires that the individual patient be tested for photosensitivity before treatment. In efforts to establish tests that reliably predict a clinical response, numerous investigators have tried to correlate blood concentrations of

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