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Antigenic similarity between the protein neurotoxin α-bungarotoxin and neuromuscular blocking drugs

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Abstract

The snake neurotoxins α-bungarotoxin (α-BGT) and β-bungarotoxin (β-BGT) which act at the neuromuscular junction, were found to bind to IgE antibodies directed against neuromuscular blocking (NMB) drugs in the sera of two patients who had experienced lifethreatening anaphylactic reactions to succinylcholine. α-BGT inhibited IgE-binding to a choline-Sepharose solid support in one patient better than the NMB drug alcuronium, choline, triethylcholine and β-BGT. IC_{50}s for α-BGT and succinylcholine were 16 and 10 nmol respectively for one patient and 34 and 6.0 nmol for the other.

Recognition of the NMB drugs and α-BGT by the same antibody is the first demonstration of an antigenic similarity between these drugs and the protein toxin.

Introduction

Elapid snake neurotoxins are known to bind specifically and with high affinity to acetylcholine receptors on muscle cells [1–6] and the electroplax of various electric fishes [7, 8]. α-Bungarotoxin (α-BGT), an extensively studied basic polypeptide neurotoxin of 74 amino acid residues and MW 7983 [9] from *Bungarus multicinctus*, is a postsynaptic or curaremimetic neurotoxin which blocks the nicotinic acetylcholine receptor at the motor end plate and produces a non-depolarising block of neuromuscular transmission [10]. Competitive neuromuscular blocking (NMB) drugs like d-tubocurarine also act at the postjunctional membrane thereby blocking competitively the transmitter action of acetylcholine [11]. Since venom neurotoxins have a higher affinity for the acetylcholine receptor ($K_D \sim 10^{-11}$ *M*, [12]) than the NMB drugs, they are consequently more toxic.

While investigating lifethreatening anaphylactoid reactions to NMB drugs administered to patients during anaesthesia, we found significant levels of IgE antibodies that reacted with these drugs in the sera of many of these patients [13–16]. Inhibition experiments revealed that these antibodies cross-react with compounds containing tertiary and quaternary ammonium groups. Drugs found to inhibit include the NMB drugs, alcuronium, d-tubocurarine, pancuronium, succinylcholine, decamethonium, gallamine, atracurium, metocurine, vecuronium, fazadinium, pharmacologically-unrelated drugs such as morphine, neostigmine, pentolineum, trimethaphan and other alkylquaternary ammonium compounds not used as muscle relaxants [13,16–19].

Since both α-BGT and competitive NMB drugs are recognised by, and hence bind to the same α-subunit of the acetylcholine receptor, we reasoned that the drug-reactive IgE antibodies in the sera of NMB drug-sensitive patients may bind to α-BGT. To investigate whether such binding occurs *in vitro*, we performed comparative inhibition experiments with NMB drugs and with α-and β-BGTs.

Materials and methods

Subjects and sera

Patients who experienced lifethreatening anaphylactic reactions following administration of a NMB drug were bled within one month of experiencing the reaction.

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Reagents

α-Bungarotoxin, β-bungarotoxin, cytochrome C from horse heart, bovine α-lactalbumin, bovine serum albumin, choline chloride (Sigma Chemical Co., U.S.A.); alcuronium dichloride (Hoffman-La Roche, Basel, Switzerland); d-tubocurarine chloride, decamethonium bromide and succinylcholine chloride (Wellcome Australia, Australia); pancuronium bromide (Organon Oss, Holland); gallamine triethiodide (May and Baker, Australia); bovine β-lactoglobulin (Koch-Light Laboratories, England); triethylcholine iodide was synthesised as previously described [16].

Drug-Sepharose solid supports

Alcuronium-Sepharose [17], d-tubocurarine-Sepharose [14], vecuronium-Sepharose [18], choline-Sepharose and triethylcholine-Sepharose [16] were prepared as previously described.

Inhibition assay

Serum (50 ul) appropriately diluted, was incubated for 1 h with 50 ul of a solution of the compound being tested before addition of the solid phase (6 mg in 100 ul). After 3 h, the tubes were washed and centrifuged three times with phosphate-buffered saline-Tween 20 (0.1%) before the addition of ^{125}I-rabbit-anti-human IgE (2×10^4 cpm/tube; Pharmacia). After overnight incubation at room temperature, the tubes were washed three times then counted in a Packard auto-gamma spectrometer.

Results

The Table summarises data obtained from inhibition experiments where the sera from 2 of 16 patients who reacted clinically to NMB drugs were preincubated with α-BGT, β-BGT, 6 NMB drugs, choline and triethylcholine. Figs 1 and 2 show inhibition results with the 2 reactive sera, Pe and Lo respectively, with choline-Sepharose as the solid-support. No, or weak inhibition, with the snake toxins was observed when these 2 sera were used in inhibition assays with other solid supports including alcuronium-, d-tubocurarine-, vecuronium- and triethylcholine-Sepharose. From the data, it is clear that choline-reactive IgE antibodies in the sera of both patients recognised both BGTs as well as choline, the 6 NMB drugs and triethylcholine.

With patient Pe who reacted clinically to the NMB drug succinylcholine, but had previously reacted to decamethonium, the most potent NMB drug inhibitor of IgE-binding to choline-Sepharose was d-tubocurarine (0.72 nmol for 50% inhibition). Alcuronium proved to be the weakest drug inhibitor requiring 58 nmol for

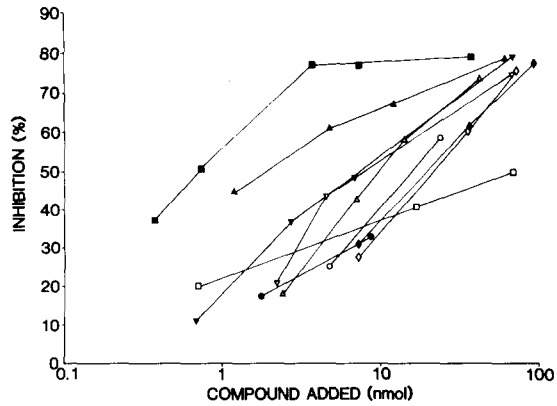

Figure 1
Inhibition by NMB drugs, choline, triethylcholine and snake bungarotoxins of IgE antibody-binding to choline-Sepharose in patient Pe. Serum Pe was used at a dilution of 1:8.

Key to symbols:
(○) α-BGT
(●) β-BGT
(□) alcuronium
(■) d-tubocurarine
(△) succinylcholine
(▲) decamethonium
(▽) gallamine
(▼) pancuronium
(◇) choline
(◆) triethylcholine

50% inhibition (Table and Fig. 1). α-BGT was intermediate in inhibitory potency requiring 16 nmol of toxin to produce 50% inhibition. β-BGT was not as potent an inhibitor (6.4 nmol for 30% inhibition; 50% inhibition not achieved) as α-BGT, making the latter a more potent inhibitor

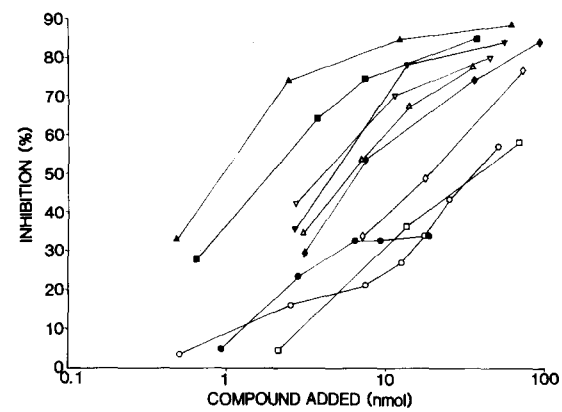

Figure 2
Inhibition of IgE antibody-binding to choline-Sepharose in patient Lo. Serum Lo was used at a dilution of 1:2.
Key to symbols:
(○) α-BGT
(●) β-BGT
(□) alcuronium
(■) d-tubocurarine
(△) succinylcholine
(▲) decamethonium
(▽) gallamine
(▼) pancuronium
(◇) choline
(◆) triethylcholine

Table 1
Cross-reactivity of α-BGT and β-BGT with neuromuscular blocking drugs: inhibition studies with sera containing IgE antibodies to succinylcholine and decamethonium

Serum*	Drugs eliciting adverse reaction	Amount (nmol) of compound needed for 50% inhibition									
		α-BGT	β-BGT	succinylcholine	decamethonium	alcuronium	d-tubocurarine	gallamine	pancuronium	choline	triethylcholine
Pe	succinylcholine/ decamethonium	16	>9.0**	9.8	1.9	58	0.72	8.4	7.7	23	20
Lo	succinylcholine	34	>18**	6.0	0.89	37	1.8	4.0	4.7	18	6.8

*Serum Pe and Lo were used at dilutions of 1:8 and 1:2 respectively.
**With serum Pe, 30% inhibition with 6.4 nmol. With serum Lo, 30% inhibition with 5.0 nmol. Fifty percent inhibition not achieved with both sera.
The proteins cytochrome C from horse heart, α-lactalbumin, β-lactoglobulin and bovine serum albumin did not inhibit at concentrations up to 30, 30, 25 and 25 nmols respectively.

than alcuronium, choline, triethylcholine and β-BGT (Table and Fig. 1). Unfortunately, the very small quantity of serum Pe available prevented more extensive investigations of inhibition of IgE-binding by α-BGT and β-BGT.

With patient Lo who reacted clinically to succinylcholine, decamethonium was the strongest inhibitor of IgE-binding (0.89 nmol for 50% inhibition). α-BGT proved to be a more potent inhibitor (34 nmol for 50% inhibition) than alcuronium and β-BGT (at concentrations above 18 nmol). Between 1.4 nmol and 18 nmol, β-BGT inhibited more strongly than α-BGT, although no significant increase in inhibition was observed at concentrations above 6.4 nmol with β-BGT (Table and Fig. 2).

Four proteins, cytochrome C from horse heart (MW 13,370 daltons), α-lactalbumin (14,500 daltons), β-lactoglobulin (37,100 daltons) and bovine serum albumin (67,000 daltons) at concentrations up to 30, 30, 25 and 25 nmol respectively did not inhibit binding of IgE antibodies in sera Pe and Lo to choline-Sepharose.

Discussion

The site of action of competitive NMB drugs (of which curare is the classic example) and the snake neurotoxin α-BGT, is the 40,000 dalton alpha unit on the postjunctional acetylcholine receptor [20, 21]. As the site and mode of action of both classes of compounds are similar, we investigated the possibility that drug-reactive IgE antibodies in allergic patients who reacted clinically to NMB drugs may cross-react with α-BGT. As the Table and Figs 1 and 2 show, cross-reactivity was demonstrated with 2 sera and the α-toxin, as well as the β-toxin, inhibited IgE-binding. In fact, with serum Pe, α-BGT inhibited IgE-binding to choline-Sepharose better than choline, triethylcholine and the NMB drug alcuronium (Table and Figs 1).

Molecular models of the 6 NMB drugs tested here have revealed that despite their differences in MW and chemical composition, the intra-quaternary or tertiary nitrogen distance is similar for each compound [22] and the substituted ammonium ions are quite accessible for antibody binding [16, 19]. In previous studies, we have demonstrated that the cross-reactivity that occurs with the drug-reactive IgE antibodies in allergic patients' sera is due to the quaternary and tertiary ammonium groups on the drug molecules which are the allergenic determinants [13–19].

The question must then be asked how the proteins, α-BGT and β-BGT (MW 21,800; [23]), can be recognised by, and consequently bind to, IgE antibodies directed against tertiary or quaternary ammonium ions on small structurally-unrelated molecules of MW less than 800. One possible explanation is based on size rather than chemical composition. Competition between a polypeptide and a small ligand at a protein binding site has been previously reported with nucleases [24] and proteases [25]. CHANGEUX *et al.* [7] postulated that the respective sizes of α-BGT and the NMB drug d-tubocurarine might not exclude the possibility of such competitive binding at the cholinergic receptor site. Assuming that α-BGT is a sphere of density 1.3, CHANGEUX *et al.* point out that the expected diameter of the molecule would be 27 Å. The distance between the quaternary and tertiary nitrogens in d-tubocurarine, a semi-rigid molecule, is approximately 14 Å [22]. On a size-basis alone then, these two compounds may be able to bind to the same antibody combining site just as they both bind to the same area of the cholinergic receptor macromolecule. However, α-toxins in general have been shown to be polypeptides with a flat shape revealed by X-ray crystallography [26–28]. It has been reported that there exist common structural features of the three-dimensional organisation of erabutoxin from *Laticauda semifasciata* with quaternary ligands but, striking structural differences between snake α-toxins and quaternary ligands are recognised [12].

WANG *et al.* [29] investigated the affinity of various ligands for the isolated α-BGT binding sites from muscle and optic lobe of the chick. They found that NMB drugs including d-tubocurarine, gallamine and decamethonium (which had the greatest affinity for muscle α-BGT binding sites of all compounds tested) had some of the highest affinities for the α-BGT binding sites. In fact, these NMB drugs bound with greater affinity than both nicotine and acetylcholine. These findings suggest that NMB drugs present a conformational shape and charge that is complimentary to the α-BGT binding sites and therefore probably similar to that presented by α-BGT. IgE-antibody recognition of the muscle relaxant drugs on the one hand

and α-BGT on the other, appear to demonstrate an antigenic similarity between the NMB drugs and the protein toxin.

Although β-BGT also inhibited IgE-binding to choline-Sepharose, this toxin is apparently not closely related structurally to α-BGT. β-BGT (MW 21,800) consists of two subunits of MW 8,800 and 12,400 held together by disulfide bonds [23]. However, the relationships between the different BGTs, α, β, χ and P-4 is not clear and amino acid sequence data is incomplete [9, 23, 30, 31]. In addition, the mode of neuromuscular blocking action of α-BGT and β-BGT is quite different. Whereas α-BGT acts postsynaptically by binding to the acetylcholine receptor and producing a non-depolarising block of neuromuscular transmission, β-BGT acts presynaptically by inhibiting the release of acetylcholine and is known not to bind to acetycholine receptors at motor end plates [11]. However, since both toxins and the NMB drugs inhibited choline-reactive IgE antibodies, it would appear that the two preparations share an antigenic similarity.

Of 16 patients investigated, only 2 were found to bind IgE antibodies that reacted with the snake BGTs. Given the known heterogeneity of antibodies to the same antigen [32] at the class, subclass and combining site levels, this is not necessarily a surprising finding. It is already clear that IgE antibodies to the same NMB drug show significant specificity differences [17].

It is interesting to note that with the other drug-solid phase supports investigated including alcuronium-, d-tubocurarine-, vecuronium- and triethylcholine-Sepharose, little or no inhibition of IgE-binding with sera Pe and Lo was observed with both BGTs. Inhibition by α-BGT and β-BGT of IgE-binding to choline-Sepharose was observed with one other patient (Hu; data not shown) who reacted clinically to alcuronium. Both toxins weakly inhibited the Hu IgE antibodies, producing less than 25% inhibition at concentrations of 30 nmol. With sera Pe and Lo (Figs 1 and 2), a definite similarity in the order of inhibitory potency of the compounds tested emerged. For serum Pe, the order of decreasing inhibitory potency was d-tubocurarine, decamethonium, pancuronium, gallamine, succinylcholine, α-BGT, triethylcholine, choline, alcuronium and β-BGT while for serum Lo, the order was decamethonium, d-tubocurarine, pancuronium and gallamine, succinylcholine, triethylcholine, choline, α-BGT, alcuronium and β-BGT. These findings suggest that the specificity of the antibody combining site in the sera of both patients is similar. It should also be noted that both patients reacted clinically to the same drug, succinylcholine. In patient Hu, the order of potency was again similar to that found in Pe and Lo (decamethonium, d-tubocurarine, succinylcholine, gallamine, pancuronium, alcuronium, choline, triethylcholine, β-BGT and α-BGT). On the other hand, sera from other patients who reacted clinically to succinylcholine or other NMB drugs, but whose IgE did not bind α-BGT or β-BGT, exhibited quite different profiles of inhibitory potencies.

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