

Allergy to Muscle Relaxants

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INTRODUCTION

With the now widespread and extensive use of muscle relaxant drugs in general anesthesia, a steadily increasing number of cases of anaphylactic-like reactions is being reported (1–3). Muscle relaxants seem to be responsible for half of the adverse reactions occurring during general anesthesia (4). The muscle relaxants predominantly implicated are *d*-tubocurarine, alcuronium, gallamine, suxamethonium, pancuronium, vecuronium, and more recently atracurium. Alcuronium is mostly involved in Australia, whereas anaphylactic shocks are rather provoked by suxamethonium in France. Clinical symptoms vary from mild cutaneous erythema to major life-threatening cardiovascular collapse and/or bronchospasm. It is clear that the well-known histamine-releasing properties of neuromuscular blocking agents cannot account for all adverse reactions observed. Fifty percent of subjects who experienced anaphylaxis after the administration of muscle relaxants may be sensitive to one or more of these agents. In these cases, classical evidence of Type I hypersensitivity (i.e., anaphylaxis) is found, and these patients usually demonstrate exquisite intradermal sensitivity to the injection of dilute relaxant solutions. The presence of seric-specific IgE against muscle relaxants has been demonstrated, thus providing an opportunity to look for serological crossreactivity by using the compounds in radioimmunoassay inhibition experiments. The antigenic group of muscle relaxants lies in the quaternary or tertiary ammonium group. These groups also confer neuromuscular-blocking properties

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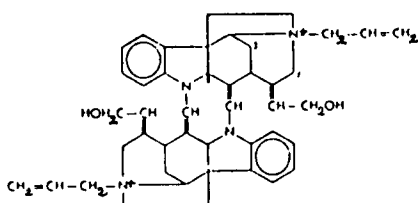
upon the drug, and their distance apart is appropriate to bridge IgE molecule and produce anaphylactic reaction.

During these last years, the role of muscle relaxants in the occurrence of allergic reactions during general anesthesia has been studied: clinical reactions, risk factors, diagnostic tools, mechanisms of this reaction at cellular and seric levels, and the role of the quaternary ammonium ion determinants.

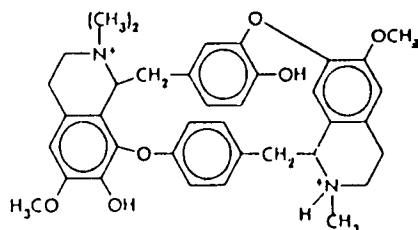
MUSCLE RELAXANTS USED DURING GENERAL ANESTHESIA

As shown in Fig. 1, muscle relaxants are very small molecules that contain quaternary ammonium ions. It is generally assumed that drugs cannot be true allergens if they are not linked to some "carriers." However, such drugs, called "mirror molecules," have at least two haptenic determinants in their structure and may act as true allergens. Muscle relaxants can bridge IgE antibodies through the ammonium ion determinants (5).

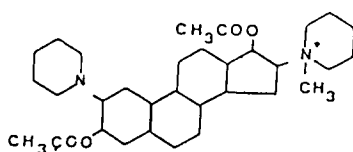
Suxamethonium is a flexible molecule, in which the two ammonium ions are separated by eight carbons and two oxygens leading to a maximum chain length of 11.6 Å. Suxamethonium can be viewed as two molecules of choline linked by a succinic acid. Pancuronium consists of a steroidal framework bearing two quaternary ammonium ions separated from each other by a distance of 10.8 Å, with a proximal phenyl ring structure. Vecuronium is identical to pancuronium, but bears only one tertiary ammonium ion. Both drugs are more rigid and hydrophobic than suxamethonium. Gallamine is a trivalent molecule. The distance between the ammonium quaternary ions is 10.7 Å. Alcuronium and *d*-tubocurarine are divalent molecules with rigid structure. Alcuronium contains two quaternary bridge *N* atoms, each linked to an allylic substituent; *d*-tubocurarine contains two-ring *N* atoms, one present as a quaternary ammonium ion with two attached methyl groups and the other as a tertiary ammonium ion with one methyl substituent. Atracurium is a new competitive neuromuscular blocking drug, structurally different from the commonly used muscle relaxant drugs; it nevertheless still contains two quaternary ammonium groups, which are accessible to antibody binding.



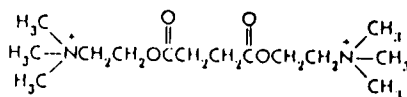
Alcuronium



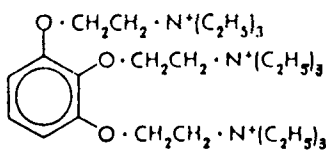
d-Tubocurarine



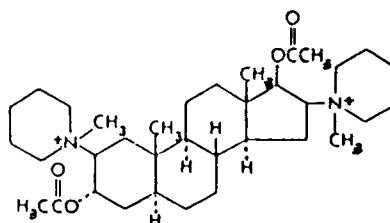
Vecuronium



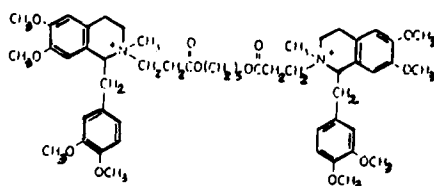
Succinylcholine



Gallamine



Pancuronium



Atracurium

Fig. 1 Structural formulae of muscle relaxants.

CLINICAL ALLERGIC REACTIONS AND RISK FACTORS

Anaphylactic reactions with tachycardia, vascular collapse, and cutaneous reactions (flush, edema, urticaria) are the most frequent adverse reactions, but some other symptoms, such as bronchospasm, diarrhea, and coagulation disorders, can be observed. In a series of 36 patients with general allergic reaction following injection of muscle relaxants, collapse was observed 30 times, urticaria and edema 20 times, bronchospasm 7 times, and cardiac arrest 3 times. These clinical features are about the same as those observed in a previous series of 41 patients (6). Fisher reported a 37% incidence of bronchospasm accompanying anaphylaxis, and a 90% incidence of hypotension and circulatory collapse (7). In a group of 34 patients investigated by Moneret-Vautrin and colleagues, the cardiovascular collapse was observed in all cases with cutaneous reactions involving erythema or Quincke's edema in 80% of cases and bronchospasms in 39% of cases (8). The outcome of allergic reaction is mainly dependent on the severity of the cardiovascular consequences and on appropriate shock treatment with perfusion of plasma substitutes, oxygen, and adrenalin. Despite appropriate treatment, about 6% of the patients who suffered such reactions died (9).

A basic question is to know whether atopy predisposes individuals or affects the severity of an anaphylactic reaction owing to muscle relaxants. Most of what we know regarding risk factors for drug allergy has been derived from studies of allergy to the penicillins. However, it is not obvious that the principles involved in penicillin allergy could be applicable to other allergenic drugs as well. In the past years, several studies have been devoted to the relationship between allergy to muscle relaxants and atopy (2,10-16). Most of these studies have demonstrated a higher percentage of atopy in patients with drugs allergy than in controls. However, because the incidence is low, the presence of such a history is not a reliable predictor of the likelihood of a reaction in an individual patient, and does not indicate either that the patient should be investigated or pretreated, or that the selection of drug be altered to reduce the likelihood of a reaction (17). Indeed, there is a lack of precise epidemiological studies, and the question of the possible role of atopy as a predisposing factor to allergy to muscle relaxants remains open depending on the definition of "atopy." We have shown that atopy,

Table 1
Concentration of Agents Used in Skin Testing

Agent	Stock reagent concentration, mg/mL	Minimal dilution and agent concentration for specific positive skin test, µg/mL	
Suxamethonium	10	1:100	100
Pancuronium	2	1:10	200
Vecuronium	4	1:10	400
Gallamine	20	1:100	200
Alcuronium	5	1:100	50
<i>d</i> -Tubocurarine	3	1:1000	3
Atracurium	10	1:1000	10

defined based on clinical and/or biological ground, is not an important risk factor for the occurrence of anaphylactic reactions (18).

In most publications, the incidence of adverse reactions to muscle relaxants was higher in women (6,10,15). This observation has not been satisfactorily explained, and raises the problem of genetic factor and/or sensitization. All risk factors are discussed elsewhere in this issue on allergy and anesthesiology.

MECHANISMS OF ANAPHYLACTIC REACTIONS TO MUSCLE RELAXANTS

Skin Tests

Skin testing with muscle relaxants is not only a reliable method of detecting allergy, but also provides great insight into the underlying mechanisms (19–21). Patients can be extremely sensitive: We recorded positive skin tests for some patients with doses of suxamethonium as low as 0.01 µg/mL. In normal volunteers, doses 10–10⁵ times higher were necessary to elicit positive skin tests (6).

Skin tests were performed on the volar surface of the forearm by intradermal injection of 0.02 mL of serial tenfold dilutions of muscle relaxants. End-point titration was calculated, and results were read at 15 min. A wheal and flare lasting more than 9 and 20 mm, respectively, were regarded as a positive reaction. Positive cutaneous reactions were considered as specific if they occurred at a drug concentration that gave negative results for all control subjects tested (Table 1), i.e., 100 µg/mL for suxamethonium, 200 µg/mL

for pancuronium and gallamine, 400 $\mu\text{g/mL}$ for vecuronium, 50 $\mu\text{g/mL}$ for alcuronium, 3 $\mu\text{g/mL}$ for *d*-tubocurarine, and 10 $\mu\text{g/mL}$ for atracurium. Normal saline without preservatives was used as a negative control and codein phosphate as the positive control (6,19,22).

Prick tests carried out on the anterior part of the forearm with commercial solutions of muscle relaxants were recently proposed (23). The correlation with intradermal skin tests is excellent; they are equally sensitive (over 95%) and absolutely specific except for atracurium. They are also reproducible (24). In few cases, it is possible to find negative prick tests to vecuronium and pancuronium in allergic patients. For these two drugs, we have to use an intradermal skin test at a dilution of 1:10, i.e., 400 $\mu\text{g/mL}$ and 200 $\mu\text{g/mL}$, respectively.

Several correlations were found between the physical signs noted during anaphylaxis and the results of skin testing. Patients who had experienced multiple physical signs during their anaphylactic events were more likely to present positive skin tests than were patients with an isolated physical sign. The diagnostic utility of skin testing has been strongly emphasized (19–25). Skin testing has been considered to be of primary diagnostic utility in IgE-mediated phenomena, especially those involving intact protein antigens, and is more sensitive than leukocyte histamine release or specific IgE detection (6,26,27). Crossreactivity between muscle relaxants, especially between suxamethonium and gallamine, has been reported by several authors. Such crossreactivity is the result of the presence of ammonium ions in the molecule (Fig. 1). Few reports have been published about reproducibility of tests used for diagnosis of drug allergy (28). In the case of penicillin allergy, skin tests tended to become rapidly negative (29). The results appear very different from those we have obtained with suxamethonium. The reproducibility of positive or negative skin tests with muscle relaxant after 1–4 yr indicates that these tests are worthwhile (30,31). The persistence of skin reaction to suxamethonium could be owing to the nature of its antigenic determinants. The quaternary ammonium ions are widespread among commonly used products, such as cosmetics, antiseptics, detergents, and the membranes of bacteria and parasites. Occasional contacts with these quaternary ammonium com-

ponents could thus maintain sensitivity to suxamethonium or other muscle relaxants without administration of the drugs.

Finally, although there is no doubt about the usefulness of skin tests for diagnostic purposes, their predictive value is uncertain. The question is to know if it is necessary to perform skin tests to muscle relaxants in patients without a history of allergic reaction during a previous anesthesia or before a first anesthesia. The probability of finding a positive skin test is evaluated in a range of 1 out of 8000 patients tested, considering the incidence of systemic reaction resulting from muscle relaxants and providing that a general reaction is always associated with a positive skin test. However, it is not proved that a positive skin test will not be systematically followed by a systemic reaction. Furthermore, the possibility of either false positive or false negative skin tests must be considered. This prevents their systematic use without raising disproportionately their cost. Nevertheless, the existence of a positive skin test indicates a risk factor and represents an indication for the eviction.

Detection and Specificity of IgE Antibodies

Detection of IgE Antibodies

The presence of IgE antibodies was suggested by passive transfer tests and leukocyte histamine release (6). However, direct proof of the presence of IgE antibodies was brought about by an Australian group (26,32) who, by covalently coupling alcuronium and *d*-tubocurarine, found high levels of drugs-reactive IgE antibodies in some subjects hypersensitive to these muscle relaxants. Solid-phase complexes of alcuronium and *d*-tubocurarine (33) were prepared by covalently coupling the drugs to activated Sepharose. Because the other commonly used muscle relaxants do not contain groups that may be exploited for coupling to a solid phase, suitable structural analogs were sought. This resulted in the selection of choline to detect antibodies to succinylcholine and decamethonium, triethylcholine for gallamine-reactive antibodies (34), and vecuronium for antibodies that react with pancuronium (35). Specific antidrug IgEs in serum were detected by a radioimmunoassay (RIA) involving covalent bonding of the drug to Epoxy-activated Sepharose-6B (Pharmacia, Uppsala, Sweden).

Leukocytes from patients can release their histamine content specifically in the presence of muscle relaxants (6,21,36–38). Histamine release with suxamethonium, one of the most widespread muscle relaxants, is often used as a model. Typical dose–response curves with inhibition in excess of suxamethonium are observed in some patients. The addition of deuterium oxide to the medium causes a significant increase in histamine release in positive cases and induces release in majority of negative cases. In contrast with polyarginine and L-methionyl-L-leucyl-L-phenylalanine, which act by a non-IgE-dependent mechanism, suxamethonium-induced histamine release was inhibited by in vitro anti-IgE leukocyte desensitization (6). In vitro suxamethonium by itself, without any carrier in the buffer, could act as a true allergen on target leukocytes (39). Acetylcholine $\text{CH}_3\text{-COO-CH}_2\text{-CH}_2\text{N}^+(\text{CH}_3)_3$ and choline $\text{CH}_2\text{OH-CH}_2\text{N}^+(\text{CH}_3)_3$, which are monovalent regions of the suxamethonium molecule, may specifically inhibit in vitro leukocyte histamine release induced by suxamethonium. Furthermore, choline in vivo may act as a hapten inhibitor and, thus, causes negative skin tests to suxamethonium in allergic patients (40). These findings point to the terminal part of a molecule (quaternary or tertiary ammonium ions) as the haptenic determinant.

Specificity to IgE Antibodies—Inhibition Studies

Data to check the specificities of IgE antibodies were obtained from inhibition studies, in which patients' sera were preincubated with each of the muscle relaxants. Considerable variations in the potency of the muscle relaxants as inhibitors were evident with the drug support.

Quantitative inhibition studies showed that IgE antibodies from most patients crossreacted with all muscle relaxants tested and with unrelated agents containing ammonium ions (26,32–35,41): many drugs (neostigmine, pentolinium, trimethaphan, and morphine) or substances as well as cell membrane constituents contain ammonium ions. In this case, inhibition studies revealed that the binding of IgE to the drug on the drug support could be diminished or prevented by this wide variety of compounds (32). Inhibition of IgE antibodies to choline Sepharose could be obtained by the addition of increasing concentrations of either choline, suxamethonium, or the synthesized diammonium salts. For the same concentration of qua-

ternary ammonium determinant, ethamethonium $[(\text{CH}_3)_3 \text{N}^+ (\text{CH}_2)^2 - \text{N}^+ (\text{CH}_3)_3]$, which is the salt where ammonium groups are very close (3.8 Å) since they are separated only by two carbons, was the least effective in the inhibition test. On the other hand, the other salts (butamethonium = 6.2 Å, hexamethonium = 8.5 Å, octamethonium = 10.6 Å) were as effective as choline or suxamethonium (42). The way in which patients become sensitized to muscle relaxants is unknown, but in the light of these findings, it seems possible that sensitization may occur without previous exposure to muscle relaxant drugs (43).

ROLE OF QUATERNARY AMMONIUM DETERMINANTS

The role of ammonium ions in mediating allergic reactions suggested by previous findings was confirmed by subsequent experiments (6). Notably it has been shown that synthesized diammonium salts with various chain lengths according to the formula $(\text{CH}_3)_3 \text{N}^+ - (\text{CH}_2)_n - \text{N}^+ (\text{CH}_3)_3$ ($n = 2, 4, 6, 8$) can specifically inhibit the *in vitro* binding of IgE antibodies to choline Sepharose in the same way as suxamethonium (42,44). The length of the chain linking the ammonium groups seems to play an important role. In fact, when the length was 4 Å, no significant histamine release could be obtained, and the optimal length appeared to be >6 Å. Flexibility of the chain linking the hapten determinants may play an important role in the binding of two IgE molecules on the membrane of target cells. Pancuronium can bind specific IgE antibodies in the sera of patients allergic to suxamethonium, as demonstrated by radioallergosorbent test (RAST) inhibition, but still could not induce specific histamine release. Since the maximum length of chain between the ammonium groups of suxamethonium (11.6 Å) is not very different from that of pancuronium (11.8 Å), it is suggested that compounds with rigid backbone (such as pancuronium) are less active than flexible molecules (such as suxamethonium) in bridging IgE molecules and in initiating mediator release (44). These facts were already suggested in penicillin allergy, because compounds with a rigid backbone were less active in eliciting positive skin reaction in penicillin-sensitive individuals (45).

However, in one case we observed a patient who had experienced an anaphylactic shock to pancuronium with a positive skin

test to the drug, but negative skin test to suxamethonium, which also has two quaternary ammonium ions and furthermore is a more flexible molecule. Direct RAST was positive for pancuronium (uptake of ^{125}I anti-IgE >12%), but negative for suxamethonium. Inhibition RAST studies demonstrated in the patient's serum the presence of IgE antibodies to pancuronium, which do not react with other substances containing quaternary ammonium ions, such as suxamethonium and choline, or with the rigid steroidal backbone of pancuronium, without ammonium ions, as androstenediol. Histamine release induced by pancuronium and JLK 204 (an analog of a muscle relaxant in which the two ammonium ions are separated by a flexible chain of six carbon atoms and linked to a phenyl ring) is significantly positive, whereas suxamethonium did not induce histamine release. Our data suggest that, in some cases, allergy to muscle relaxants does not involve ammonium ions alone and may be related to the presence of IgE with a specificity to some other determinants.

Baldo and Fisher described three main types of sensitization (32). The sera of some patients recognize all muscle relaxants sharing the quaternary ammonium equally well. Other patients' sera recognize all chemical myorelaxant structures, but with large differences in the degree of RAST inhibition. Finally, some patients' sera recognize only one family of muscle relaxants. For such patients, the influence of the microenvironment of the quaternary ammonium ion on recognition by specific IgE antibodies must be taken into account. In this regard, it is important to mention the results of Pallardy et al. (46), who raised polyclonal IgG antibodies against eliptinium acetate, a molecule that also presents a quaternary ammonium ion. These results underline the importance of the counter-ion of the ammonium ion, since the chloride form is 10 times less recognized than the acetate form.

Concerning the functional features of IgE involved in allergy to muscle relaxants, three points should be emphasized. The first is that most muscle-relaxant-allergic subjects exhibit IgE antibodies with high specificity for quaternary and tertiary ammonium ions. The second is that length and flexibility of the chain seem to play a major role in the bridging of IgE antibodies on the target cells. The third is that, in some cases, other structures, e.g., phenyl

rings, may also be important for recognition, although quaternary and tertiary ammonium groups are probably the principal regions of the drugs recognized by complementary IgE antibodies. Indeed, in some individuals, the nature of the group surrounding the quaternary ammonium ion may be the most important factor in recognition by the IgE antibodies.

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