**Clinical Practice**

Differentiating the cellular and humoral components of neuromuscular blocking agent-induced anaphylactic reactions in patients undergoing anaesthesia

R. C. Aalberse 1*, I. Kleine Budde 1, M. Mulder 1, S. O. Stapel 1, W. Paulij 2, F. Leynadier 3 and M. W. Hollmann 4

1 Department of Immunopathology, Sanquin Research, Plesmanlaan 125, 1066CX Amsterdam, The Netherlands
2 MSD, Oss, The Netherlands
3 Centre d’Allergologie, Hôpital Tenon (AP-HP), Paris, France
4 Department of Anesthesiology, Academic Medical Center, Amsterdam, The Netherlands
* Corresponding author. E-mail: r.aalberse@sanquin.nl

**Editor’s key points**
- Neuromuscular blocking agents are the main cause of anaphylaxis during anaesthesia.
- Both IgE and cellular components appear to be involved but skin-prick and serum tests give inconsistent results.
- Only two of 23 rocuronium IgE-positive serum samples were able to sensitize stripped basophils.
- There was a correlation between IgE to rocuronium and skin-test reactivity to succinylcholine.
- The authors conclude that both IgE reactivity and altered cellular reactivity are required for anaphylaxis.

**Background.** The significance of IgE antibodies to neuromuscular blocking agent (NMBA)-induced anaphylactic reactions during anaesthesia is unclear. We investigated the relevance of IgE to rocuronium using an in vitro technique.

**Methods.** Serum samples from 61 patients with anaphylactic reactions during anaesthesia were investigated. On the basis of clinical history, allergy to NMBA was considered likely in 48 patients, further assessed using intradermal skin tests for several commonly used NMBA, including rocuronium, vecuronium, and succinylcholine. To determine the presence of rocuronium IgE in human serum, a rocuronium-human serum albumin (rocHSA) conjugate was coupled to a solid phase and a radioallergosorbent test performed. The biological effects of patient serum NMBA-IgE on histamine release were investigated using in vitro sensitized basophils from healthy blood donors.

**Results.** IgE to rocuronium was found in 23 of 48 serum samples (48%) with NMBA allergy, although only two of these were able to sensitize basophils to release histamine in response to rocHSA. IgE-responsiveness in the basophil test was only observed with conjugated rocHSA and not with unconjugated rocuronium or the other NMBA evaluated. However, unconjugated rocuronium inhibited the histamine release induced by rocHSA. Correlation between skin-test reactivity to rocuronium and IgE to rocHSA was low (P>0.1). In contrast, striking correlation between IgE to rocuronium and skin-test reactivity to succinylcholine was found (P<0.001).

**Conclusions.** Our results indicate that NMBA-related anaphylaxis requires not only IgE NMBA reactivity, but also altered cellular reactivity in the patient. The latter may be demonstrable by testing basophils from the patient, a skin test with (steroidal) NMBA, or both.

**Keywords:** neuromuscular block, allergy; neuromuscular block, rocuronium; neuromuscular block, succinylcholine

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The incidence of anaphylactic reactions (immune- or non-immune-mediated) in anaesthesia has been reported to be between one in 6000 and one in 20 000 procedures,1 2 with neuromuscular blocking agents (NMBA) implicated as the main cause.1 3 However, the real incidence remains inadequately characterized. Skin-prick or intradermal tests are commonly used to identify the probable drug involved, while drug-induced activation of patient basophils has successfully been used to confirm the diagnosis.3 Indeed, NMBA-induced basophil reactivity was reported in 17 of 47 patients (36%) with proven NMBA anaphylaxis, with positive predictive value increasing to 86% (six of seven patients) for those tested within 3 yr.4 NMBA-induced anaphylaxis is associated with an IgE-dependent mechanism in ~60% of cases.3 A French study of the prevalence of NMBA sensitivity found that 24 of 258 patients (9.3%) were reactive to NMBA; 12 had positive skin-prick tests and the serum from 14 contained IgE antibodies but only two subjects were positive in both tests.7 The prevalence of reactivity to quaternary ammonium groups, the key antigenic determinants of NMBA, is
substantially higher in the general population than the incidence of anaphylactic reactions during anaesthesia, suggesting that an NMBA-induced anaphylactic reaction requires a combination of humoral (NMBA-reactive IgE antibody) and cellular factors (altered mast cell reactivity). Allergic reactivity to conventional allergens, such as grass pollen allergens, can be transferred via serum. An in vitro alternative is the stripped basophil histamine release assay. In this assay, basophils are stripped of their own IgE, and then sensitized by the patient serum, thereby avoiding the effects of changed reactivity in the patient’s cells.

The aim of the current study was to evaluate the role of cellular factors and IgE to NMBA in anaphylactic events during surgery using the stripped basophil test in conjunction with the results of intradermal skin tests with NMBA.

Methods

The study was approved by the local medical ethics committee at the Hôpital Tenon, Paris, France, and conducted in accordance with the Declaration of Helsinki.

Serum samples were collected at the Hôpital Tenon, Paris, France, and other hospitals from patients with anaphylactic reaction during surgery [defined according to clinical criteria, ranging from cutaneous signs (flushing, rash, urticaria) with hypotension associated with unexplained tachycardia to life-threatening symptoms, including cardiovascular collapse, bronchospasm, hypoxia, angiooedema, and cardiac arrest with or without cutaneous symptoms]. In patients agreeing to return for a follow-up skin test, diagnosis of NMBA involvement was evaluated based on clinical data (i.e. timing of event relative to NMBA administration and known allergy to other products used during surgery) and skin tests by an investigator who was blinded to serology results. All patients agreed to serum being collected for analysis, which was immediately frozen at –80°C and stored for future analysis.

For the intradermal skin tests, a volume of 0.03–0.05 ml of test solution was injected into the forearm and the wheal and erythema diameter measured 20 min later. The test was judged to be positive if the diameter of the wheal was at least 80% of the positive control (histamine 10 mg ml⁻¹). NMBA intradermal skin tests were performed at the following dilutions: succinylcholine 1/100, 1/1000 (Celocurine®, Sanofi-Synthelabo, Paris, France; 1/100=200 μg ml⁻¹, 554 μM), rocuronium 1/100, 1/1000 (Esmeron®, MSD, Oss, The Netherlands; 1/100=100 μg ml⁻¹, 164 μM), pancuronium 1/10, 1/100, 1/1000 (Pavulon®, MSD; 1/10=200 μg ml⁻¹, 273 μM), vecuronium 1/10, 1/100, 1/1000 (Nocuron®, MSD; 1/10=200 μg ml⁻¹, 314 μM), and atracurium 1/10, 1/100, and 1/1000 (Tracrium®, GlaxoSmithKline, Greenford, UK; 1/100=100 μg ml⁻¹, 80.4 μM). The higher concentrations were only tested if there was a negative skin test with a more dilute solution. The results were expressed as \(-\log_{10}\) of the threshold concentration, i.e. 2+ indicated a skin test positive at 1/100 but negative at 1/1000 dilution and 3+ indicated a skin test positive at 1/1000 dilution. Steroidal drugs were grouped into one class and any patients scoring a positive reaction to any member of this class were considered as fulfilling the inclusion criterion for the study.

Conjugation of rocuronium bromide

To couple rocuronium to a solid phase suitable for IgE measurements, rocuronium bromide (supplied as a powder by MSD) was coupled to human serum albumin (HSA) (18 mol per mol HSA) via the 3α-hydroxyl group of the androsane structure using carbodiimide-mediated esterification to produce the rocuronium bromide conjugate, rocHSA. As a negative control, an androstane derivative of rocuronium, without the morpholinyl and propenyl pyrrolidinium group (17b-acetyloxy-3α-hydroxy-5α-androstane, supplied by MSD) was also conjugated to HSA (15.6 mol per mol HSA), referred to as androstaneHSA.

Radioallergosorbent test

The CAP system (Pharmacia, Uppsala, Sweden) was used to determine the serum concentration of IgE to succinylcholine. IgE to rocHSA was determined using the radioallergosorbent test (RAST) as, at the time of the study, no suitable reagent was available for the CAP system, although one has subsequently been developed.

For the RAST, rocHSA and androstaneHSA (equivalent to 5 mg HSA) were added to 300 mg cyanogen bromide-activated Sepharose (Pharmacia, Uppsala, Sweden), and suspended at 2 mg ml⁻¹ in phosphate-buffered saline (PBS) with 0.3% HSA, 0.01 M EDTA, 0.05% sodium azide (NaN₃), and Tween 20 (PBS-AT). This results in the binding of >80% of the added HSA conjugate.

The RAST was performed as described previously. Briefly, serum (50 μl) from patients and control subjects without history of anaphylaxis was incubated overnight with rocHSA- or androstaneHSA-Sepharose (250 μl). IgE serum levels >2500 IU ml⁻¹ can produce false-positive results; therefore, if the total IgE level was >2500 IU ml⁻¹, the serum was mixed with human serum with low total IgE (<2 IU ml⁻¹) to decrease the IgE concentration to 2000 IU ml⁻¹. After washing five times, ¹²⁵I-labelled sheep anti-human IgE (see below) was added together with 500 μl PBS, with 100 μg ml⁻¹ HSA, 20 μg ml⁻¹ sheep immunoglobulin (purified using caprylic acid as described by McKinney and Parkinson), 0.1% Tween 20, and 0.06% NaN₃. The suspension was then incubated overnight. After washing four times, the Sepharose-bound radioactivity was determined, expressed as a percentage of the total counts added. Negative control monoclonal mouse/human chimeric IgE antibody to the house dust mite allergen Der p 2 (IgE 2000 IU ml⁻¹) did not result in non-specific IgE binding for any NMBA.

Affinity-purified sheep anti-human IgE (M1294, Sanquin, Amsterdam, The Netherlands) was labelled using the ¹²⁵I chloramine-T method, using HSA as a carrier protein instead of bovine serum albumin. Labelled anti-IgE was diluted in PBS with 0.3% HSA, 0.01 M EDTA, and 0.05% NaN₃ and stored at –20°C. After correction for radioactivity...
bound to the buffer control, the results were converted to international units of IgE per millilitre using chimeric IgE antibody as a reference. If applicable, these results were corrected for non-specific binding to a negative-control Sepharose. If the corrected IgE value was < 0.3 IU ml\(^{-1}\) or < 1.5 times the baseline value, the results were considered to be negative. If the blank-corrected IgE value was between > 0.3 and < 1.0 IU ml\(^{-1}\), the result was classified as borderline, but for the \(\chi^2\) statistic, IgE antibody results < 1 IU ml\(^{-1}\) were scored as negative. A blank-corrected IgE value of \(\geq\) 1.0 IU ml\(^{-1}\) was considered positive. Positive RAST to rocHSA was confirmed at least once.

**RAST inhibition**
The specificity of NMBA-IgE positive sera (rocHSA and succinylcholine) was tested in an inhibition RAST. Before addition of rocHSA-Sepharose (0.5 or 2 mg test), serum was incubated with 100 \(\mu\)l of an NMBA-containing agent [rocuronium, succinylcholine (Suxamethonium, Pharmachemie), rocHSA, and androstaneHSA] diluted with an equal volume of 0.1 M phosphate buffer (pH 7.2) for 2 h. The RAST was repeated as described above. To exclude RAST inhibition by a non-specific effect, grass pollen-positive serum was pre-incubated with the NMBA and subsequently tested in the grass pollen RAST. No non-specific inhibition was found. On the basis of the standard error, a substance was considered to inhibit a serum RAST if pre-incubation of the serum and substance resulted in a decrease in the raw RAST score by 2\% of added radioactivity.

**Histamine release bioassay**
To circumvent effects due to variable activity of nicotinic acetylcholine receptors on basophilic leucocytes, the biological effects of NMBA-IgE were investigated using \textit{in vitro} sensitized basophils from selected, consenting healthy blood donors at the Department of Plasmapheresis, Sanquin (Amsterdam, The Netherlands). The buffy coats were derived (Haemonetics Plasma Collection System, Haemonetics Corporation, Braintree, MA, USA) using 1000 ml of blood from each donor. The basophils were enriched (2–5\% purity) using Percoll centrifugation (1.078 g cm\(^{-3}\)) and the histamine release assay was performed as described previously. Three assay runs were performed, in which each serum sample was tested twice. Lactic acid buffer (pH 3.9) was used to remove IgE from the surface of the basophils and the cells were then sensitized by incubation (37 °C, 90 min) with a ‘sensitization mixture’ containing 150 \(\mu\)l human serum, 4 mM EDTA, and 10 \(\mu\)g ml\(^{-1}\) heparin in a total volume of 1 ml. Because of the limited amount of serum available, sensitization with serum from the anaphylactic patient group was performed using a total volume of 333 \(\mu\)l. The sensitized cells (2.5–0.8 \(\times\) 10\(^6\) in a final volume of 350 \(\mu\)l) were challenged in the presence of 1 mM CaCl\(_2\) by adding rocHSA, free rocuronium, or a control and incubated for 60 min at 37 °C. For each serum sample, an allergen, anti-IgE, or both were used as a positive control for sensitization of IgE. The negative control was HEPES buffer with interleukin-3 [IL-3; 20 mM HEPES, 132 mM NaCl, 6 mM KCl, 1 mM CaCl\(_2\), 1 mM MgSO\(_4\), 1.2 mM K\(_2\)HPO\(_4\), 5.5 mM glucose, 5 mg ml\(^{-1}\) HSA, pH 7.4 and 0.6 mM IL-3 (Pepro-Tech Inc., Rocky Hill, NJ, USA)]. The controls gave good responses (histamine release > 15\%). The histamine release reaction was stopped by the addition of 750 \(\mu\)l of ice-cold normal saline. Positive serum samples were confirmed in four further experiments.

To assess inhibition of histamine release, rocHSA or allergen extract was diluted in different concentrations of NMBA [rocuronium, vecuronium, pancuronium, mivacurium (Mivacron\(^\text{®}\), GlaxoSmithKline), or succinylcholine] adjusted to pH 7.4. Histamine release was determined by fluorimetric analysis and calculated as the percentage of total cellular histamine content, determined by perchloric acid cell lysis. Histamine release values were corrected for non-specific histamine release; the lowest value either from basophils sensitized by IgE-deficient serum or from the serum of an NMBA-negative atopic subject. If percentage histamine release after correction was below 5\%, the reactivity was considered negative; between 5\% and 10\%, the result was classified as borderline and additional testing was needed. A score of > 10\% was interpreted as definite IgE reactivity. The background fluorescence signal of the investigational product in buffer was subtracted from the histamine release of the sample before this percentage was determined. If this signal was > 10\% of the total histamine fluorescence signal in the cell sample, a specific response < 10\% was considered negative. Both atracurium (di)besilate and cisatracurium (di)besilate (Nimbex\(^\text{®}\), GlaxoSmithKline) showed an unacceptably high background in our histamine assay and were not evaluated.

**Statistical analysis**
Associations were analysed using \(\chi^2\) with Yates’ correction for continuity, and by Spearman’s correlation. For the \(\chi^2\) statistic, IgE antibody results were scored as negative if the antibody level was < 1 IU ml\(^{-1}\).

**Results**

**Patients allergic to NMBA**
Of 63 patients assessed, 61 provided serum samples and sufficient information to ascertain that an anaphylactic reaction had occurred during anaesthesia. In total, 58 patients had skin tests for succinylcholine, vecuronium, and/or pancuronium; of these, 26 (45\%), 27 (47\%), and 25 (43\%), respectively, were graded as \(\geq 2+\). Skin tests for rocuronium were performed in 42 patients, 13 (31\%) of whom were graded as \(\geq 2+\).

On the basis of intradermal skin testing and clinical evaluation, allergy to NMBA was considered likely in 48 of the 61 patients (79\%) (Table 1). Most (81\%) were female, mean age at skin testing was 49.8 yr (range 28–75 yr), and the time between anaphylactic reaction and skin testing ranged from 0 to 19 yr. During surgery, the NMBA given were: vecuronium (n = 14), succinylcholine (n = 10), rocuronium (n = 10), pancuronium (n = 3), atracurium (n = 4),
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<td>IgE† rocHSA</td>
<td>0.1</td>
<td>34.9</td>
<td>2.1</td>
<td>0.1</td>
<td>0.1</td>
<td>2.3</td>
<td>76.9</td>
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<td>0.1</td>
<td>4.1</td>
<td>4.1</td>
<td>0.1</td>
<td>1.0</td>
<td>2.7</td>
<td>1.5</td>
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<tr>
<td>IgE† succinylcholine</td>
<td>4.2</td>
<td>34.5</td>
<td>34.6</td>
<td>0.1</td>
<td>0.1</td>
<td>2.7</td>
<td>1.0</td>
<td>0.1</td>
<td>6.7</td>
<td>2.5</td>
<td>6.0</td>
<td>2.6</td>
<td>21.3</td>
<td>0.3</td>
<td>1.2</td>
<td>1.6</td>
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<tr>
<td>IgE† phosphorylcholine</td>
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<td>0.5</td>
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<tr>
<td>IgE† airborne allergen</td>
<td>1.9</td>
<td>0.5</td>
<td>0.1</td>
<td>1.9</td>
<td>0.1</td>
<td>1.4</td>
<td>0.5</td>
<td>0.1</td>
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<td>1.7</td>
<td>0.1</td>
<td>0.8</td>
<td>1.0</td>
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<td>Total IgE†</td>
<td>540</td>
<td>5423</td>
<td>43</td>
<td>56</td>
<td>32</td>
<td>194</td>
<td>1091</td>
<td>6</td>
<td>177</td>
<td>205</td>
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<td>330</td>
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<td>18</td>
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gallamine (n=3), mivacurium (n=1), and unknown NMBA (n=3). NMBA involvement was insufficiently substantiated in the remaining 13 patients (21%) for the following reasons: no NMBA was used, lack of response to the intradermal tests, or known allergy to latex or an antibiotic.

**Prevalence of NMBA-specific IgE and its relation to skin-test reactivity**

After correction (androstaneHSA: highest IgE level 1.1 IU ml\(^{-1}\)), 23 of the 48 serum samples (48%) were positive for IgE to rocHSA (Table 1; Fig. 1). Several samples (six of 99) from the control group were also positive for IgE to rocHSA (Fig. 1). Thirty-one of the 48 serum samples (65%) were positive for IgE to succinylcholine, and 28 to phosphorylcholine (58%) (Table 1). Correlation between the concentration of IgE to rocHSA and IgE to succinylcholine showed that of the 48 patients, 21 were positive to rocHSA and succinylcholine, 10 only to succinylcholine, and two only to rocHSA (Fig. 2).

**Basophil histamine release**

Of the samples positive/borderline for IgE to rocHSA in the RAST (i.e. samples with IgE \(>0.3\) IU ml\(^{-1}\); n=25), only two, S1 and S30, were able to sensitize basophils to release histamine to rocHSA. Basophils sensitized with S1 required a high concentration of rocHSA (optimal concentration 232 \(\mu\)M; calculation based on rocuronium) to release histamine, whereas the optimal concentration for basophils sensitized with S30 was substantially lower (0.2–2.0 \(\mu\)M rocHSA) (Fig. 3).

Basophils sensitized with serum samples, S1 and S30, did not release histamine in response to unconjugated rocuronium, vecuronium, pancuronium, succinylcholine, or...
mivacurium. The positive controls used for sensitization of IgE (allergen and/or anti-IgE) were associated with a good response (histamine release >15%). Neither rocuronium nor the rocHSA conjugate had an effect on non-sensitized basophils or on activation of basophils sensitized by IgE (with a specificity unrelated to rocuronium) and challenged with the appropriate allergen.

**Inhibition of histamine release**

The histamine release induced by rocHSA from basophils sensitized by serum samples S30 (Fig. 4) and S1 could be inhibited by unconjugated rocuronium. Notably, to inhibit the histamine release in response to rocHSA, a 10-fold higher concentration of unconjugated rocuronium was needed for basophils sensitized by serum sample S1 compared with cells sensitized by S30. Vecuronium and pancuronium also abolished histamine release induced by rocHSA from basophils sensitized by S30, whereas neither mivacurium nor succinylcholine had inhibitory effects. AndrostaneHSA did not influence histamine release for either serum sample. There was no evidence of non-specific inhibition, since unconjugated rocuronium did not inhibit the reaction induced by mite allergen in control bioassays.

**Specificity of IgE to rocuronium determined by RAST inhibition**

IgE cross-reactivity between rocHSA and succinylcholine was investigated in four serum samples (S1, S30, S48, S63) based on their rocHSA IgE content and whether they were positive (S1, S30) or negative (S48, S63) in the histamine release assay in response to rocHSA (Table 2).

More efficient RAST inhibition by succinylcholine was expected in serum sample S1 (low rocHSA IgE, high succinylcholine IgE levels) compared with serum sample S30 (high rocHSA IgE, low succinylcholine IgE levels). However, in all four serum samples, inhibition with unconjugated rocuronium (tested at 5.5 mM) was greater than with succinylcholine (tested at 18.5 mM) (Table 2). Pre-incubation with androstaneHSA did not affect IgE binding in the tested samples. There was no evidence of non-specific RAST inhibition, with pre-incubation with grass pollen-positive serum.

**Level of association between NMBA-specific IgE and skin-test reactivity**

The association between the presence of IgE to rocHSA and skin-test reactivity to rocuronium was low: 12 of 30 patients (40%) in the skin-test-negative group were positive or borderline positive for IgE to rocHSA vs nine of 13 patients.
Study of NMBA-induced anaphylactic reactions

In this study, we examined the mechanisms involved in NMBA-induced anaphylactic reactions, and also the underlying mechanisms of tests utilized in current diagnoses. The likely cause of the anaphylactic reaction was determined using intradermal skin tests for several commonly used NMBA, using the dilutions recommended in guidelines to avoid false-positive results.19–21 None of the four patients in whom atracurium was implicated had a positive response at the recommended 1/1000 dilution. Nevertheless, these patients were included in the study because they had a positive response to at least two other NMBA s and/or because the timing of the event relative to atracurium suggested NMBA involvement and other potential causes were excluded.

Rocuronium and succinylcholine were each used in only 10 of the 48 cases in our study. However, in agreement with previous reports of cross-reactivity between NMBA s,19 about half of the samples were positive for IgE to rocuronium and 65% for IgE to succinylcholine. Most of the positive samples (21/33) had IgE to both rocuronium and succinylcholine and only two to rocuronium alone.

A substantial fraction of our natural antibody repertoire is reactive with quaternary ammonium groups, including acetylcholine-related substances such as NMBA and phosphorylcholine, a far more ubiquitous compound. These antibodies are inducible by exposure to environmental factors, microbial (e.g. pneumococcal), and parasitic antigens, and may be cross-reactive with some commonly used chemicals (detergents, shampoos, toothpastes),19 22 and other quaternary ammonium ion-containing drugs.23 As anaphylactic responses to NMBA s have occurred in patients who have not received an NMBA before,24 it is likely that most NMBA-reactive antibodies are not initially induced by NMBA exposure.25 Indeed, we found IgE to rocuronium in samples taken before rocuronium was introduced to the market. Cross-reactive antibodies are expected to have a relatively low affinity for NMBA, offering a plausible explanation for the lack of biological relevance in some cases.

Succinylcholine and rocuronium IgE cross-reactivity was assessed in our study using four samples selected for their IgE content. Even in samples with a much greater succinylcholine IgE content than rocuronium IgE content, pre-incubation with unconjugated rocuronium resulted in a greater inhibition of IgE binding in the rocuronium RAST than pre-incubation of (69%) in the skin-test-positive group (P>0.1). The association between IgE to rocuronium and skin-test reactivity to succinylcholine (Spearman’s ρ=0.53; 95% confidence interval: 0.29–0.71, P<0.001) was higher than with skin test reactivity to rocuronium (Spearman’s ρ=0.13; 95% confidence interval: 0.21 to 0.43, P>0.1; Fig 5). The two patients (serum samples S1 and S30) with a positive basophil test to rocuronium were exceptional in this respect, because both had a negative skin test with succinylcholine (despite the presence of IgE reactive with succinylcholine). Whereas unconjugated rocuronium did not activate basophils sensitized by S1 and S30 to release histamine upon challenge with rocuronium, the skin test with unconjugated rocuronium (1/1000 dilution) was positive in the patient from whom serum sample S30 was derived (no rocuronium skin test was performed in the patient for serum sample S1).

Discussion

In this study, we examined the mechanisms involved in NMBA-induced anaphylactic reactions, and also the underlying mechanisms of tests utilized in current diagnoses. The likely cause of the anaphylactic reaction was determined using intradermal skin tests for several commonly used NMBA s, using the dilutions recommended in guidelines to avoid false-positive results.19–21 None of the four patients in whom atracurium was implicated had a positive response at the recommended 1/1000 dilution. Nevertheless, these patients were included in the study because they had a positive response to at least two other NMBA s and/or because the timing of the event relative to atracurium suggested NMBA involvement and other potential causes were excluded.

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with succinylcholine. This suggests that, while there is cross-reactivity between the NMBA IgE, there are differences in sensitivity. Surprisingly, we showed that correlation between skin test reactivity to rocuronium and presence of IgE to rocHSA was low. In contrast, skin test reactivity to succinylcholine was closely associated with IgE reactivity (not only to succinylcholine, but also to rocuronium).

Interestingly, only two of the IgE-positive serum samples were able to sensitize stripped basophils to release histamine in response to rocHSA, and neither sample sensitized

![Fig 5](https://academic.oup.com/10.1093/bja/aen278/article-abstract/106/5/665/279260)
basophils to release histamine in response to unconjugated rocuronium or the other NMBA s. This indicates that the stripped basophil test is unsuitable for diagnosis of NMBA-related anaphylactic reactions. This is not because of an intrinsic lack of diagnostic sensitivity of the test, as histamine release in the bioassay has been shown to have good correlation with IgE levels in patients with allergy to house dust mites. On the basis of this previous experience, we expected 15 of the 48 current samples to have a positive response to rochHSA in the stripped basophil bioassay. The reason for this discrepancy is not clear, especially given the close association between IgE serology and skin test reactivity to succinylcholine, but it suggests that IgE to NMBA s does not have the same properties as IgE to protein allergens.

Differences in affinity cannot be the sole explanation for the discrepancies between IgE antibody measurements and biological reactivity, particularly for those patients with a positive skin test to rocuronium without obvious specific IgE. The two serum samples with a positive histamine release result cannot be used as an illustration of an NMBA-induced allergic reaction because we found basophil activation only with conjugated rocuronium (rochHSA), whereas non-conjugated rocuronium was ineffective and actually inhibited activation by the conjugate. This indicates that rocuronium acts as a monovalent hapten, whereas anaphylactic activity requires the NMBA to be at least divalent.

The current study (Fig. 1) and others showed a substantially increased prevalence of NMBA-reactive IgE in patients with anaphylaxis during surgery compared with the general population and patients with other allergies, and thus it is likely to play some pathogenic role. We hypothesize that some NMBA-reactive cellular component is hyperactive, which is sufficient in some cases to cause an IgE-independent, non-immune-mediated anaphylactic reaction. In other cases, collaboration with NMBA-reactive IgE may be needed. This cellular hyperactivity is likely to be expressed in tissue cells and blood basophils, as suggested by the high prevalence of basophil activation in patients with anaphylaxis during surgery. One interesting possibility for the contrasting correlation data is that the skin test to succinylcholine reflects immunological hyper-reactivity to acetylcholine-related structures, whereas skin-test reactivity to pancuronium and vecuronium (and similar NMBA s, but not succinylcholine) reflects cellular hyper-reactivity. In future studies, it will be important simultaneously to compare in vivo sensitized basophils and stripped normal basophils sensitized with IgE from serum obtained from the same patients.

Measurement of tryptase levels in the current study would also have provided important information on whether the anaphylactic reaction in these patients involved mast cell activation. Unfortunately, this was not possible because all the serum samples were obtained some weeks after the anaphylactic reaction and tryptase levels are only meaningful if samples are obtained within a few hours of the event.

In conclusion, this study provides further information on mechanisms underlying the current diagnostic procedures for suspected NMBA-induced anaphylactic reactions. The analytical protocol for NMBA-related anaphylactic events should (in addition to measurement of plasma tryptase) include two types of diagnostic test: (i) a test to establish the presence of IgE reactive to succinylcholine; and (ii) a test to establish cellular hyper-reactivity. The first test could be a skin test with succinylcholine, IgE serology, or both while the second test may comprise a skin test with a steroidal NMBA (e.g. pancuronium), and/or an ex vivo cellular test using basophils obtained from the patient before, or a few weeks after, the anaphylactic event.

Acknowledgement
The authors would like to thank Professor David Levy (Paris) for his help in preventing information getting lost in translation.

Conflict of interest
W.P. is an employee of MSD, Oss, The Netherlands. R.C.A., S.S., I.K.B., and M.M. are employees of Sanquin, Amsterdam, The Netherlands. For the duration of this project, R.C.A. was a paid consultant to MSD.

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