

Rocuronium Hypersensitivity: Does Off-Target Occupation of the MRGPRX2 Receptor Play a Role?



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What is already known about this topic? The neuromuscular blocking agent (NMBA) rocuronium is a relevant cause of perioperative hypersensitivity. Recently, it has been suggested to reclassify hypersensitivity to NMBA as type A reactions resulting from off-target occupation of the nonimmune MRGPRX2 receptor.

What does this article add to our knowledge? According to the results of triple testing (skin tests, basophil activation test, and quantification of specific IgE) and quantitative basophil inhibition experiments, we think hypersensitivity to rocuronium mainly results from a genuine IgE/high-affinity receptor for sIgE (FcεRI)-dependent effector cell activation.

How does this study impact current management guidelines? We think hypersensitivity to rocuronium still mainly results from IgE/FcεRI-dependent effector cell activation. However, it cannot be excluded that in some patients rocuronium hypersensitivity results from off-target occupation of the MRGPRX2 receptor.

BACKGROUND: The neuromuscular blocking agent (NMBA) rocuronium is a relevant cause of perioperative hypersensitivity (POH) with a significant risk of diagnostic error. Recently, it has been suggested to reclassify hypersensitivity to NMBA as type A reactions resulting from off-target occupation of the nonimmune MRGPRX2 receptor.

OBJECTIVE: To investigate whether basophil activation experiments can benefit diagnosis and add to the insights in the pathomechanisms of rocuronium hypersensitivity.

METHODS: A total of 140 patients with a suspected POH to rocuronium in whom peak tryptase was available had complete diagnostic workup for all potential culprits including triple confirmatory testing with skin tests, basophil activation test (BAT), and quantification of specific IgE (sIgE) antibodies to rocuronium and morphine. To further analyze the clinical relevance of sIgE antibodies, quantitative basophil inhibition experiments were performed by cocubation of the cells with

rocuronium and morphine, an opiate known to harbor a substituted ammonium structure.

RESULTS: Diagnosis of rocuronium hypersensitivity was established in 72 of 140 patients (51.4%), of whom 65 (90.3%) demonstrated mast cell activation. Of the 72 patients, 64 displayed a positive skin test, 8 (11.1%) had their diagnosis documented only by BAT. Cocubation of morphine and rocuronium induced a dose-dependent inhibition of BAT with rocuronium that was restricted to 4 of 6 patients with IgE reactivity to rocuronium and/or morphine.

CONCLUSIONS: BAT can benefit diagnosis of rocuronium hypersensitivity. As basophils barely express MRGPRX2 and BAT rocuronium can be inhibited by morphine, we believe that hypersensitivity to rocuronium still mainly results from IgE/high-affinity receptor for sIgE (FcεRI)-dependent effector cell activation. However, it cannot be excluded that in a few patients rocuronium hypersensitivity results from off-target occupation of the MRGPRX2 receptor. © 2018 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2019;7:998-1003)

Key words: Basophil; CD63; Hypersensitivity; Mast cell activation; MRGPRX2; Rocuronium; Skin test; Specific IgE; Tryptase

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Abbreviations used

BAT- Basophil activation test
FcεRI- High-affinity receptor for sIgE
IDHR- Immediate drug hypersensitivity reactions
IDT- Intradermal test
MC- Mast cell
MCA⁺- Mast cell activation
MCA⁻- No mast cell activation
NAP6- National Audit Project
NMBA- Neuromuscular blocking agent
NPV- Negative predictive value
POH- Perioperative hypersensitivity
PPV- Positive predictive value
ROC^{BAT+}- Positive BAT rocuronium
ROC^{BAT-}- Negative BAT rocuronium
ROC^{BATNR}- Nonresponder in BAT rocuronium
ROC^{ST+}- Positive skin test rocuronium
ROC^{ST-}- Negative skin test rocuronium
sIgE- Specific IgE
SPT- Skin prick test

predictive value (NPV) of 96%.⁴ Alternatively, it is clear that the diagnosis of rocuronium hypersensitivity should not solely rest on a positive result for rocuronium specific IgE (sIgE) nor sIgE to morphine.⁵ These sIgE tests show too low specificity,^{4,5} mainly because of nonspecific binding to the solid phase as observed with elevated total IgE titres.⁶

At present, 10 studies have investigated the diagnostic performances of basophil activation tests (BAT) in NMBA hypersensitivity.³ Compared with skin testing traditional BAT attains a sensitivity between 36% and 92% and a specificity between 81% and 100%. For rocuronium, the BAT reaches an excellent PPV of 97% and an NPV of 75%.⁴ However, to our knowledge, there are currently no data on the added value of BAT in patients displaying equivocal or negative skin tests.

Here we sought to investigate whether BAT rocuronium could benefit diagnosis and identify patients in whom diagnosis would have been overlooked because of negative skin test investigation. Furthermore, we hypothesize on the potential of BAT and basophil coincubation experiments to further clarify on the proposal to reclassify NMBA hypersensitivity as an IgE/high-affinity receptor for sIgE (FcεRI)-independent reaction resulting from off-target occupation of the nonimmune receptor MRGPRX2.⁷

MATERIALS AND METHODS

Study population

We evaluated a total of 140 patients who were referred to our outpatients' clinic for diagnostic evaluation after experiencing a POH reaction grades 1-4 according to the National Audit Project (NAP6) severity criteria.⁸ All had rocuronium as NMBA and quantification of peak serum tryptase within 90 minutes after onset of their reaction. All patients underwent a standardized protocol for all potential offenders of perioperative anaphylaxis.⁹ With respect to rocuronium, all patients underwent skin testing, BAT, and quantification of sIgE to rocuronium but also sIgE morphine,⁴ the latter being a marker for sensitization to tertiary and quaternary substituted ammonium structures.^{6,10,11}

Confirmatory testing for rocuronium

All individuals had skin tests with the aminosteroid (rocuronium, Esmeron; Merck Sharp and Dohme, Brussels, Belgium), negative control (saline buffer), and a positive control (10 mg/mL histamine; HAL Allergy Benelux NV, Haarlem, The Netherlands). Skin tests included skin prick tests (SPT) and, if negative, intradermal tests (IDT). Maximal test concentration was 10 mg/mL (undiluted) for SPT and 0.05 mg/mL (dilution 1/200) for IDT.¹² For the SPT and the IDT, rocuronium was diluted immediately before use. SPT with a wheal ≥ 3 mm with surrounding erythema after 15 minutes were considered positive. For IDT, injection of 0.05 mL was performed and reactions were read after 20-30 minutes. IDT responses with a wheal and flare ≥ 8 mm (or doubling of injection bleb) were considered positive.

The BAT for rocuronium is described in detail elsewhere.¹³ Results were expressed as the net percentage of CD63+ basophils and threshold of positivity was set at 4%.¹³

sIgE to rocuronium and morphine was quantified by the ImmunoCAP system (Phadia Thermo Fisher, Uppsala, Sweden) according to the manufacturer's instructions. For rocuronium, decision threshold was set at 0.13 kU_A/L and for morphine at 0.35 kU_A/L.⁶

To elucidate on the clinical relevance of sIgE rocuronium antibodies, in a separate set of experiments, we compared the effect of basophilic coincubation with morphine and anti-IgE (positive control) as well as morphine and rocuronium between 6 patients with positive sIgE results and 3 patients with negative sIgE results to rocuronium and/or morphine.

Mast cell activation (MCA) was defined as a peak value exceeding $1.2 \times$ baseline tryptase + 2, as recently validated in POH.¹⁴

RESULTS

Figure 1 displays the different patients' groups according to the presence or absence of MCA and outcomes of skin testing and BAT. In total, 140 rocuronium-exposed patients who experienced a POH reaction grade 1-4 underwent a standardized diagnostic protocol for all potential culprits. All diagnostics were performed between 7 and 3672 days after the index reaction. One hundred and six cases had displayed MCA (MCA⁺), whereas 34 did not (MCA⁻).

From the 106 MCA⁺ patients, 57 had a positive skin test to rocuronium (MCA⁺ROC^{ST+}), 49 had a negative skin test for rocuronium (MCA⁺ROC^{ST-}). From the 57 MCA⁺ROC^{ST+} patients, 30 displayed a positive BAT for rocuronium (52.6%, MCA⁺ROC^{ST+BAT+}), 19 a negative BAT rocuronium (MCA⁺ROC^{ST+BAT-}), and 8 were nonresponders to stimulation with both the positive control anti-IgE and the drug (MCA⁺ROC^{ST+BATNR}). From the 49 MCA⁺ROC^{ST-} patients, 8 had a positive BAT for rocuronium (MCA⁺ROC^{ST-BAT+}). The details of these 8 MCA⁺ROC^{ST-BAT+} patients are summarized in Table I. Thirty MCA⁺ROC^{ST-} patients had a negative BAT (MCA⁺ROC^{ST-BAT-}), and 11 were nonresponsive to positive control stimulation (MCA⁺ROC^{ST-BATNR}). In the 8 MCA⁺ROC^{ST-BAT+} patients, no other cause was identifiable, suggesting a causative role for rocuronium. In contrast, in all of the 11 nonresponders and 19 of the 30 MCA⁺ROC^{ST-BAT-} patients, another culprit was identified, mainly the β -lactam antibiotic cefazolin and chlorhexidine. Only 2 of the 11 MCA⁺ROC^{ST-BAT-} who had no clear diagnosis established demonstrated sensitization to substituted tertiary and quaternary

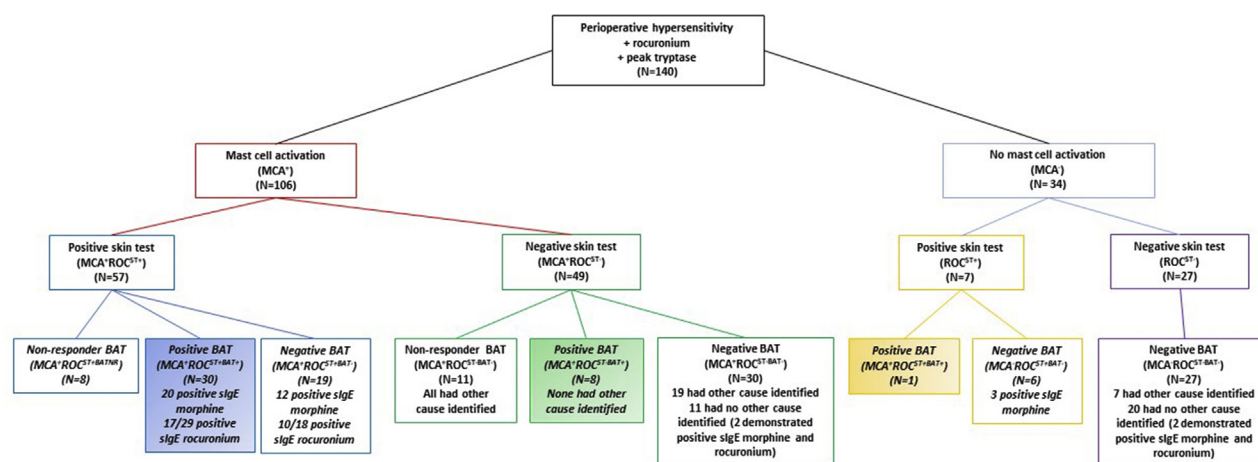


FIGURE 1. Flowchart displaying the presence or absence of mast cell activation (MCA), skin test, basophil activation test (BAT), and specific IgE (sIgE) results. MCA^+ , Mast cell activation; MCA^- , no mast cell activation; ROC^{BAT+} , positive BAT rocuronium; ROC^{BAT-} , negative BAT rocuronium; ROC^{BATNR} , nonresponder in BAT rocuronium; ROC^{ST+} , positive skin test rocuronium; ROC^{ST-} , negative skin test rocuronium.

TABLE I. Characteristics of patients displaying negative rocuronium skin tests but positive BAT

PT	Sex	Age (y)	Grade NAP6	Tryptase ($\mu\text{g/L}$)		BAT (%CD63 ⁺ cells)		Total IgE (kU/L)	ROC sIgE (kU _A /L)	MOR sIgE (kU _A /L)	INT (ds)
				Peak	Basal	algE	ROC 500				
1	F	51	4	17.6	2.7	23	10	97	<0.10	2.60	12
2	F	55	3	185	7.2	32	6	78	<0.10	4.94	92
3	M	58	3	34	14.5	58	17	69	0.21	2.41	127
4	F	58	3	18.8	5.2	38	21	NA	<0.10	<0.10	71
5	M	38	4	49.7	7.6	3	12	162	0.26	5.70	63
6	F	65	3	16.5	5.1	66	38	36	<0.10	<0.10	241
7	F	50	3	24.6	8.1	18	7	53	0.28	<0.10	89
8	F	51	3	17.6	2.4	52	41	52	<0.10	3.95	30

Severity grading according to NAP6.⁸

Peak and basal tryptase. BAT with algE and ROC 500 $\mu\text{g/mL}$; threshold for ROC was set at 4%.¹³

For ROC decision threshold was set at 0.13 kU_A/L and for morphine at 0.35 kU_A/L.⁶

Mast cell activation was defined according to the consensus formula: peak tryptase exceeding $1.2 \times$ baseline tryptase + 2 $\mu\text{g/L}$.¹⁴

algE, Anti-IgE; BAT, basophil activation test; basal, baseline; INT, interval elapsed between index reaction and diagnostic testing; MOR sIgE, morphine-sIgE; NA, not available; NAP6, National Audit Project; ROC, rocuronium; ROC sIgE, rocuronium-sIgE.

ammonium structures, as indicated by a sIgE to morphine of 7.39 kU_A/L and 0.47 kU_A/L. In these patients, total IgE was 304 kU/L and 21 kU/L, respectively.

From the 34 patients without MCA (MCA^-), 7 had a positive skin test for rocuronium (MCA^-ROC^{ST+}), of whom only 1 (14.3%) had a positive BAT ($MCA^-ROC^{ST+BAT+}$) and 3 demonstrated a positive sIgE to morphine. The remaining 6 MCA^-ROC^{ST+} , all responsive to positive control stimulation, had a negative BAT rocuronium ($MCA^-ROC^{ST+BAT-}$). All 27 MCA^-ROC^{ST-} patients also had a negative BAT for rocuronium ($MCA^-ROC^{ST-BAT-}$). In 20 of these 27 $MCA^-ROC^{ST-BAT-}$ patients, no cause was delineable. Two of these 20 $MCA^-ROC^{ST-BAT-}$ patients without demonstrable cause demonstrated sensitization to substituted tertiary and quaternary ammonium structures, as indicated by a sIgE to morphine of 0.87 kU_A/L and 0.61 kU_A/L. In these patients, total IgE was 50 kU/L and

193 kU/L, respectively. Figure 1 also displays the number of positive sIgE results in the different patients' groups.

Overall, 72 of 140 patients (51.4%) were diagnosed as hypersensitive to rocuronium, of whom 8 (11.1%) had negative skin tests and their diagnosis documented by the BAT (see Table I for individual results). Alternatively, a total of 25 ROC^{ST+} patients with responding basophils displayed a negative BAT, with 15 (60%) demonstrating a positive sIgE to morphine (12 MCA^+ , 3 MCA^-).

Table II and Figure 2 summarize the demographics, biological findings, and the results of the basophil incubation experiments. Incubation of morphine and rocuronium induced a significant dose-dependent inhibition of BAT with rocuronium that was restricted to 4 of the 6 patients with IgE reactivity to rocuronium and/or morphine. No effect was demonstrable in the patients with negative sIgE results. No effect of morphine was demonstrable on the BAT with anti-IgE.

TABLE II. Characteristics of patients undergoing basophil coinubation experiments

PT	Sex	Age (y)	Grade NAP6	Tryptase (μg/L)		BAT (%CD63 ⁺ cells)		Total IgE (kU/L)	ROC sIgE (kU _A /L)	MOR sIgE (kU _A /L)	INT (ds)
				Peak	Baseline	algE	ROC 500				
1	M	54	3	NA	NA	91	92	370	0.65	5.96	45
2	M	48	3	NA	NA	96	95	379	2.2	11	344
3	F	58	3	24.8	4.1	29	64	NA	0.15	1.09	55
4	F	51	3	44	5.5	92	63	266	0.6	1.22	55
5	F	51	3	126	6.13	57	55	78	0.04	0.14	38
6	M	35	3	200	5.29	56	44	473	0.30	33	34
7	M	22	3	30	10	11	23	181	0.03	0.05	7
8	F	34	4	200	6.2	14	22	213	2.44	2.53	43
9	M	67	4	48	7.6	43	63	243	0.11	0.43	165

Severity according to NAP6.⁸

Peak and baseline tryptase. BAT with algE and ROC 500 μg/mL; threshold for ROC was set at 4%.¹³

For ROC decision threshold was set at 0.13 kU_A/L and for morphine at 0.35 kU_A/L.⁶

Mast cell activation was defined according to the consensus formula: $1.2 \times \text{baseline} + 2 \mu\text{g/L}$.¹⁴

algE, Anti-IgE; BAT, basophil activation test; bas, baseline; INT, interval elapsed between index reaction and diagnostic testing; MOR sIgE, morphine-sIgE; NA, not available; NAP6, National Audit Project; ROC, rocuronium; ROC sIgE, rocuronium-sIgE.

DISCUSSION

To our knowledge, this is the first study to endorse the complementary value of the BAT in the diagnosis of rocuronium hypersensitivity. From our results, it emerges that, although hypersensitivity to rocuronium is more prevalent in the MCA⁺ group, it is certainly not restricted to patients who demonstrate MCA. As a consequence, diagnostic workup with confirmatory testing should not be restricted to the MCA⁺ group but also involve the MCA⁻ group. In a majority of our patients, the diagnosis of rocuronium hypersensitivity is established by skin testing, confirming that this diagnostic procedure merits the status of the primary diagnostic approach.³ However, 11% of our patients demonstrate negative skin test responses and have their diagnosis of rocuronium hypersensitivity finally established by BAT (and most also by sIgE). Although, for obvious reasons, it is impossible to perform full-dose challenges with this NMBA, we are confident that the positive BAT results in our skin test negative patients are clinically significant and indicate a genuine sIgE/FcεRI-dependent rocuronium hypersensitivity. As a matter of fact, as already exemplified in the introductory paragraph, the BAT rocuronium has an excellent PPV,⁴ and has been integrated in the diagnostic approach of rocuronium hypersensitivity in patients who display negative or equivocal skin test results.³

Alternatively, it is confirmed that BAT can be negative in ROC^{ST+} patients. Although it is likely that negative BAT outcomes mainly result from a nonresponder status of the cells or a lower test sensitivity of BAT, it is not excluded that the ROC^{ST+BAT-} status of some patients might have an alternative explanation. Actually, in ROC^{ST+} patients, negative BAT results might relate to the existence of an alternative pathomechanism of rocuronium hypersensitivity that is independent from IgE/FcεRI cross-linking.⁷ In 2015, McNeil et al¹⁵ demonstrated that engagement of the Mas-related G-protein receptor MRGPRX2 might be implicated in mast cell (MC)-driven immediate drug hypersensitivity reactions (IDHR) that are phenotypically indistinguishable from IgE/FcεRI-dependent MC degranulation. Potential MRGPRX2 agonists identified in this study are the bradykinin receptor 2 antagonist icatibant, NMBA (atracurium, rocuronium), and several fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin). Recently, similar observations

were made for opioid compounds (including morphine, codeine, and different major metabolites),¹⁶ vancomycin,¹⁷ and many other antimicrobials.¹⁸ The quintessence of these studies is clear, that is, the off-target occupancy of the MRGPRX2 receptor is increasingly recognized as a novel nonimmune endotype of MC-driven IDHR. Moreover, the cellular distribution of the MRGPRX2 might explain the discrepancies between positive skin test responses and negative outcomes of BAT in our patients. As a matter of fact, as basophils, unlike cutaneous MC_{TC} (tryptase- and chymase-positive mast cells), barely express the MRGPRX2 on their surface,¹⁹ and because basophils do not respond nonspecifically to rocuronium in individuals uneventfully exposed to this NMBA,^{4,5,13} one could, like for opiates²⁰ and fluoroquinolones,²¹ speculate basophil activation experiments not only to constitute a diagnostic to document genuine IgE/FcεRI-mediated rocuronium hypersensitivity but also to be an interesting asset to further explore the putative existence of an MRGPRX2-related endotype. This particular nonimmune endotype would not only be independent of IgE/FcεRI cross-linking but also go undetected in steady-state conditions of traditional CD63-based BAT. To verify the hypothesis that noncongruent positive skin tests and negative BAT might reflect an IgE/FcεRI-independent endotype, we first compared sIgE with both rocuronium and morphine (the “marker” for sensitization to tertiary and quaternary ammonium structures) between ROC^{ST+BAT+} and ROC^{ST+BAT-} patients who exhibited MCA during their POH reaction. Secondly, we performed basophil coinubation experiments with the positive control (anti-IgE) and morphine as well as rocuronium and morphine. A dose-dependent inhibition of the BAT rocuronium by morphine would be indicative for the clinical relevance of sIgE rocuronium. Although no absolute conclusions can be drawn, the positive results for sIgE in approximately two-thirds of the patients in both groups, and the observation morphine to significantly inhibit BAT rocuronium in most patients with positive sIgE results, call for prudence on the statement that rocuronium mainly causes IgE/FcεRI-independent hypersensitivity and the proposal to reclassify these reactions as nonimmune type A reactions.⁷ Such a reclassification would refute triple positive testing (skin test, BAT, sIgE) as a proof of IgE/FcεRI-dependent

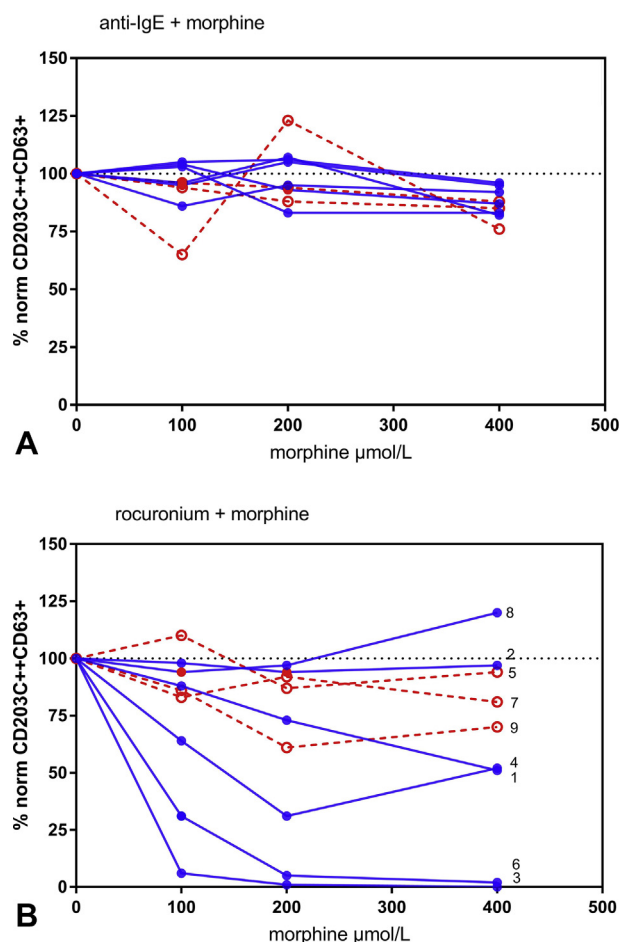


FIGURE 2. Quantitative inhibition experiments expressed as individual normalized percentages of CD203c++CD63+ basophil activation test (BAT) ($n = 9$). **A**, The BAT with the positive control anti-IgE is not affected by coincubation with morphine. **B**, In contrast, there is a dose-dependent inhibition of BAT rocuronium with morphine (0–400 $\mu\text{mol/L}$) in 4 of the 6 patients with positive (specific IgE) sIgE to rocuronium and morphine (full lines). In contrast, there is no inhibition of the BAT rocuronium in 3 patients with negative sIgE to rocuronium and morphine (dashed lines).

hypersensitivity. Moreover, an MRGPRX2-dependent mechanism is also difficult to reconcile with the observation that 11% of patients demonstrated negative skin tests but a positive BAT. *Mutatis mutandis* we call for restraint on a reclassification of hypersensitivity to other NMBA as nonimmune type A reaction. For example, for atracurium it has also been demonstrated that BAT^{22–25} and quantification of sIgE to atracurium^{10,26,27} can benefit diagnosis. Moreover, evidence has accumulated atracurium sIgE antibodies to exhibit other specificities than sIgE antibodies to rocuronium and suxamethonium.^{10,26–28} Collectively, we believe that NMBA mainly causes IgE/FcεRI-dependent hypersensitivity, but it is tempting to speculate that (some of) these drugs, in some patients, might trigger MC degranulation, by off-target occupancy of the MRGPRX2 receptor. Whether this alternative explanation applies to rocuronium remains elusive. Although significant progress has been made, in our knowledge, about NMBA-induced MRGPRX2-dependent MC

degranulation, our insights for rocuronium remain incomplete and uncertain. Actually, Lansu et al,¹⁶ although using a similar calcium imaging technique, could not confirm rocuronium to be a secretagogue agonist for MRGPRX2 in a LAD2 human MC line, as proposed by a mouse model used by McNeil et al.¹⁵ The explanation for the divergences between mice and human MC has probably to be sought in adaptive changes of the MRGPRX2 gene in human evolution,²⁹ making the human receptor more than a 10-fold less susceptible for rocuronium than its murine orthologue Mrgprb2.¹⁵ It is of note that in the recent NAP6 survey, only 4 patients who reacted to a benzylisoquinoline NMBA and none of the patients who reacted to rocuronium were classified by the authors as “nonallergic,”³⁰ as all demonstrated positive skin test and/or sIgE results.⁸ Finally, 6 years after pholcodine withdrawal, the Norwegian population has become less IgE sensitized and clinically more tolerant to NMBA.³¹

In conclusion, it is clear that rocuronium hypersensitivity cannot mainly be attributed to off-target occupancy of the MRGPRX2 receptor and that basophils and sIgE antibodies do still matter. First, the BAT is complementary to skin testing to document rocuronium hypersensitivity. Second, we believe that congruent positive triple testing (skin test, BAT, and sIgE) and the quantitative basophil inhibition data favor rocuronium hypersensitivity mainly resulting from genuine IgE/FcεRI-dependent effector cell activation. Alternatively, it should be admitted that noncongruent positive skin test and negative BAT and sIgE data could point to the existence of an alternative mechanistic endotype of rocuronium hypersensitivity independent from IgE/FcεRI cross-linking and eventually to occur as a result of off-target occupancy of the MRGPRX2 receptor. However, additional mechanistic studies in human MC and basophils are required to fill the current knowledge gaps and to enable eventual shifting of the IgE/FcεRI-MRGPRX2 paradigm for this NMBA.

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REFERENCES

- Mertes PM, Volcheck GW, Garvey LH, Takazawa T, Platt PR, Guttormsen AB, et al. Epidemiology of perioperative anaphylaxis. *Presse Med* 2016;45:758–67.
- Kemp HI, Cook TM, Thomas M, Harper NJN. UK anaesthetists' perspectives and experiences of severe perioperative anaphylaxis: NAP6 baseline survey. *Br J Anaesth* 2017;119:132–9.
- Ebo DG, Faber M, Elst J, Van Gasse AL, Bridts CH, Mertens C, et al. In vitro diagnosis of immediate drug hypersensitivity during anesthesia: a review of the literature. *J Allergy Clin Immunol Pract* 2018;6:1176–84.
- Leysen J, Bridts CH, De Clerck LS, Vercauteren M, Lambert J, Weyler JJ, et al. Allergy to rocuronium: from clinical suspicion to correct diagnosis. *Allergy* 2011;66:1014–9.
- Leysen J, Uytendaele A, Sabato V, Bridts CH, De Clerck LS, Ebo DG. Predictive value of allergy tests for neuromuscular blocking agents: tackling an unmet need. *Clin Exp Allergy* 2014;44:1069–75.
- Ebo DG, Venemalm L, Bridts CH, Degerbeck F, Hagberg H, De Clerck LS, et al. Immunoglobulin E antibodies to rocuronium: a new diagnostic tool. *Anesthesiology* 2007;107:253–9.

7. Spoerl D, Nigolian H, Czarnetzki C, Harr T. Reclassifying anaphylaxis to neuromuscular blocking agents based on the presumed patho-mechanism: IgE-mediated, pharmacological adverse reaction or "innate hypersensitivity"? *Int J Mol Sci* 2017;18:E1223.
8. Cook TM, Harper NJN, Farmer L, Garcez T, Floss K, Marinho S, et al. Anaesthesia, surgery, and life-threatening allergic reactions: protocol and methods of the 6th National Audit Project (NAP6) of the Royal College of Anaesthetists. *Br J Anaesth* 2018;121:124-33.
9. Ebo DG, Fisher MM, Hagendorens MM, Bridts CH, Stevens WJ. Anaphylaxis during anaesthesia: diagnostic approach. *Allergy* 2007;62:471-87.
10. Fisher MM, Baldo BA. Immunoassays in the diagnosis of anaphylaxis to neuromuscular blocking drugs: the value of morphine for the detection of IgE antibodies in allergic subjects. *Anaesth Intensive Care* 2000;28:167-70.
11. Laroche D, Chollet-Martin S, Leturgie P, Malzac L, Vergnaud MC, Neukirch C, et al. Evaluation of a new routine diagnostic test for immunoglobulin E sensitization to neuromuscular blocking agents. *Anesthesiology* 2011;114:91-7.
12. Mertes PM, Moneret-Vautrin DA, Leynadier F, Laxenaire MC. Skin reactions to intradermal neuromuscular blocking agent injections: a randomized multicenter trial in healthy volunteers. *Anesthesiology* 2007;107:245-52.
13. Ebo DG, Bridts CH, Hagendorens MM, Mertens CH, De Clerck LS, Stevens WJ. Flow-assisted diagnostic management of anaphylaxis from rocuronium bromide. *Allergy* 2006;61:935-9.
14. Bareto RL, Beck S, Heslegrave J, Melchior C, Mohamed O, Ekbote A, et al. Validation of international consensus equation for acute serum total tryptase in mast cell activation: A perioperative perspective. *Allergy* 2017;72:2031-4.
15. McNeil BD, Pundir P, Meeker S, Han L, Undem BJ, Kulka M, et al. Identification of a mast-cell-specific receptor crucial for pseudo-allergic drug reactions. *Nature* 2015;519:237-41.
16. Lansu K, Karpiak J, Liu J, Huang XP, McCorvy JD, Kroeze WK, et al. In silico design of novel probes for the atypical opioid receptor MRGPRX2. *Nat Chem Biol* 2017;13:529-36.
17. Azimi E, Reddy VB, Lerner EA. Brief communication: MRGPRX2, atopic dermatitis and red man syndrome. *Itch (Phila)* 2017;2:e5.
18. Zhang T, Che D, Liu R, Han S, Wang N, Zhan Y, et al. Typical antimicrobials induce mast cell degranulation and anaphylactoid reactions via MRGPRX2 and its murine homologue MRGPRB2. *Eur J Immunol* 2017;47:1949-58.
19. Sabato V, Van Gasse AL, Cop N, Claessen K, Decuyper I, Faber M, et al. The Mas-related G protein-coupled receptor MRGPRX2 is expressed on human basophils and up-regulated upon activation. *J Allergy Clin Immunol* 2017;139:AB168.
20. Leysen J, De Witte L, Sabato V, Faber M, Hagendorens M, Bridts C, et al. IgE-mediated allergy to pholcodine and cross-reactivity to neuromuscular blocking agents: lessons from flow cytometry. *Cytometry B Clin Cytom* 2013;84:65-70.
21. Van Gasse AL, Sabato V, Uyttebroek AP, Elst J, Faber MA, Hagendorens MM, et al. Immediate moxifloxacin hypersensitivity: is there more than currently meets the eye? *Allergy* 2017;72:2039-43.
22. Uyttebroek AP, Sabato V, Leysen J, Bridts CH, De Clerck LS, Ebo DG. Flowcytometric diagnosis of atracurium-induced anaphylaxis. *Allergy* 2014;69:1324-32.
23. Monneret G, Benoit Y, Debard AL, Gutowski MC, Topenot I, Bienvenu J. Monitoring of basophil activation using CD63 and CCR3 in allergy to muscle relaxant drugs. *Clin Immunol* 2002;102:192-9.
24. Kvedariene V, Kamey S, Ryckwaert Y, Rongier M, Bousquet J, Demoly P, et al. Diagnosis of neuromuscular blocking agent hypersensitivity reactions using cytofluorimetric analysis of basophils. *Allergy* 2006;61:311-5.
25. Dewachter P, Chollet-Martin S, Mouton-Faivre C, de Chaisemartin L, Nicaise-Roland P. Comparison of basophil activation test and skin testing performances in NMBA allergy. *J Allergy Clin Immunol Pract* 2018;6:1681-9.
26. Johansson SG, Oman H, Degerbeck F, Tunelli J, Florvaag E, Nopp A. Anaphylaxis to atracurium—a non-QAI-dependent reaction? *Acta Anaesthesiol Scand* 2012;56:262-3.
27. Uyttebroek AP, Sabato V, Bridts CH, De Clerck LS, Ebo DG. Immunoglobulin E antibodies to atracurium: a new diagnostic tool? *Clin Exp Allergy* 2015;45:485-7.
28. Baldo BA, Fisher MM, Pham NH. On the origin and specificity of antibodies to neuromuscular blocking (muscle relaxant) drugs: an immunochemical perspective. *Clin Exp Allergy* 2009;39:325-44.
29. Yang S, Liu Y, Lin AA, Cavalli-Sforza LL, Zhao Z, Su B. Adaptive evolution of MRGX2, a human sensory neuron specific gene involved in nociception. *Gene* 2005;352:30-5.
30. Harper NJN, Cook TM, Garcez T, Farmer L, Floss K, Marinho S, et al. Anaesthesia, surgery, and life-threatening allergic reactions: epidemiology and clinical features of perioperative anaphylaxis in the 6th National Audit Project (NAP6). *Br J Anaesth* 2018;121:159-71.
31. de Pater GH, Florvaag E, Johansson SG, Irgens A, Petersen MN, Guttormsen AB. Six years without pholcodine; Norwegians are significantly less IgE-sensitized and clinically more tolerant to neuromuscular blocking agents. *Allergy* 2017;72:813-9.