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MRGPRX2 activation in mast cells by neuromuscular blocking agents and other agonists: modulation by sugammadex by Nithya A. Fernandopulle et al.

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Conflict of interest

The authors declare no conflict of interest.

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Dear Editor,

We read with great interest the paper by Fernandopulle and colleagues about MRGPRX2 activation in mast cells (MCs) by the aminosteroid rocuronium and the benzyloquinoline atracurium and other agonists and the modulation of these responses by sugammadex ¹. However, we would like to rise some comments about their approach and interpretation of the data. Mast cell activation and degranulation via the Mas-related G protein coupled MRGPRX2 receptor provides a novel attractive paradigm in our knowledge of perioperative hypersensitivity and anaphylaxis from neuromuscular blocking agents (NMBAs).

Current evidence for this immunoglobulin E (IgE)-independent pathway of MC activation comes from animal or *in vitro* studies, and translation of the findings into clinical relevance in humans is difficult and should be critically interpreted ^{2, 3}. To the best of our knowledge, there has been only one clinical study to a better understanding of IgE- and MRGPRX2-mediated MC degranulation in response to NMBAs ⁴.

Based on the current models, the MRGPRX2-activating capacity and potency of rocuronium is left with some uncertainties. First, it appears that its MRGPRX2-activating capacity is different and difficult, if at all, predictable by its effects on its mouse orthologue. Rocuronium is ~ 12 times less potent at the MRGPRX2 receptor in humans than in mice ². Second, at least four studies have explored rocuronium-induced calcium mobilization and degranulation in different cell lines and primary human MCs ^{2, 5-7}. None of these studies disclosed MRGPRX2-dependent degranulation and calcium mobilization was only demonstrated in two of them ^{2, 7}. Actually, the MRGPRX2-dependent MC activating capacity of rocuronium could well be limited to cutaneous MCs ⁴. In the current study, the authors confirm rocuronium to enhance intracellular calcium staining but this effect is not translated in degranulation ^{2, 6, 7}. Hence, our surprise for this rocuronium-sugammadex model to further unveil the MRGPRX2-dependent MC degranulating potential of rocuronium. Besides, we have shown that in a majority of our patients, rocuronium hypersensitivity is IgE-mediated ^{4, 8}. These IgE-mediated reactions are documented by positive drug-reactive sIgE, skin tests and basophil activation tests using resting cells that do barely express MRGPRX2 ^{9, 10}. Most importantly, as MRGPRX2-dependent reactions are clinically indistinguishable from IgE-mediated reactions ⁴, we

wonder how the attending anesthetist witnessing perioperative anaphylaxis could unambiguously discriminate between both pathways. Anyhow, the conclusion sugammadex cannot be recommended in the acute management of an alleged MRGPRX2-mediated rocuronium aligns with a recent consensus recommendation ¹¹ and prior *ex vivo* findings in rocuronium allergic patients ¹².

As indicated by the authors, the modified γ -cyclodextrin sugammadex has been developed as a selective relaxant-binding agent that encapsulates steroidal NMBAs, particularly rocuronium. Both, affinity studies and *in vitro* and *in vivo* studies have demonstrated that sugammadex does not cause effective reversal of the neuromuscular blocking effects of either atracurium or cisatracurium ^{13, 14}. Therefore, the observation sugammadex to reduce atracurium-induced MRGPRX2-mediated calcium mobilization and MC degranulation is both surprising and intriguing. Especially, the finding sugammadex to be less able to inhibit the atracurium-induced calcium response compared to degranulation and cytokine release. Additional studies are required to verify the presumption sugammadex, whilst not affecting atracurium's curarizing effect, can impair its MRGPRX2-activating potential and whether biased agonism of the atracurium-sugammadex inclusion complex at the MRGPRX2 receptor can explain the divergent effects on calcium mobilization, degranulation and cytokine release. Note, however, that a concentration of 500 μ M sugammadex corresponds to the peak plasma concentration of intravenous bolus dose of sugammadex of 94 mg/kg (the maximum recommended dose by the manufacturer is 16 mg/kg). For the time being, the clinical relevance of these findings remains uncertain, just as the observation sugammadex to inhibit more delayed MRGPRX2-dependent MC responses. To the best of our knowledge, no cases of biphasic anaphylaxis from NMBAs has ever been reported, and, like with rocuronium, many patients who experienced atracurium anaphylaxis show evidence for an IgE-dependent reaction ^{15, 16}.

Finally, the authors claim that selective knocking down of the MRGPRX2 receptor via CRISPR-Cas9 gene editing is more pronounced in comparison to existing research using siRNA MRGPRX2. However, prudence should be called upon this claim, mainly because absence of comparative analyses. We have demonstrated that selective silencing of MRGPRX2 via DsiRNA electroporation coupled to flow cytometry enabled to clarify some uncertainties and unknowns in our understandings of the enthralling MRGPRX2

pathway in IDHRs. Briefly, targeting of MRGPRX2 by this technique almost completely suppressed intracellular calcium elevations in response to morphine, moxifloxacin and rocuronium, and in a lesser extent also to the neurokinin substance P (the natural ligand of MRGPRX2). Moreover, for substance P, morphine and moxifloxacin this suppressive effect was invariantly accompanied by a downscaling of degranulation ⁷.

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