The Cyclodextrin Sugammadex and Anaphylaxis to Rocuronium: Is Rocuronium Still Potentially Allergenic in the Inclusion Complex Form?

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Abstract: Rocuronium, a non-depolarizing neuromuscular blocking drug has a rapid onset of action, a comparatively low potency and, with a more favourable side effects profile than succinylcholine, it has become a popular alternative to that drug for rapid sequence inductions in anaesthesia. The rocuronium-binding cyclodextrin derivative sugammadex, prepared by per-6 substitution of the primary hydroxyls of γ-cyclodextrin with thiol ether-linked propionic acid side chains to extend the hydrophobic cavity to accommodate rocuronium, is used to reverse neuromuscular blockade by encapsulating the drug as an inclusion complex and removing it from the neuromuscular junction to the plasma. It has recently been suggested that sugammadex might also be of value in the management of rocuronium-induced anaphylaxis and this has been potentially supported by recent case reports. However, before sugammadex can be recommended for this purpose, it is important to establish whether or not the allergenic substituted ammonium groups at each end of the rocuronium molecule in the inclusion complex are masked within the cavity or left exposed for interaction with rocuronium-reactive IgE antibodies in the sera of rocuronium-allergic patients. Detailed experimental strategies and experimental protocols to investigate the allergenic potential of the sugammadex-rocuronium inclusion complex are presented and a possible explanation of the apparently rapid and successful reversal of anaphylaxis by administration of sugammadex is advanced and discussed.

Keywords: Sugammadex, perioperative anaphylaxis, cyclodextrins, drug allergy, rocuronium, drug-specific IgE.

CYCLODEXTRINS

Chemistry and Physical Form

Cyclodextrins are oligosaccharides [1-8] made up of from 6 to 13 D-glucopyranoside units [9] in rigid $^4C_1$ chair conformation [10] and linked α(1-4) in a circular arrangement around a central cavity [4,6,8,11]. The formation of these cyclic oligosaccharides occurs *via* the catalytic restructuring of starch by cyclodextrin glycosyltransferases, often of Bacilli origin [7, 12-15]. Perhaps the three most important cyclodextrins are those consisting of six, seven or eight glucopyranose units and termed, respectively, α-, β- and γ-cyclodextrin [3, 5-9,11]. The two-dimensional chemical structure of the naturally occurring γ-cyclodextrin is shown in Fig. (**1**). It has a toroidal or truncated cone shape with a total of 16 secondary hydroxyl groups on carbons two and three of each glucopyranose unit at the wider or so-called secondary rim (or face) end and eight primary hydroxyls on carbon six at the narrow or primary rim end [8,14,16]. The number of glucosidic units determines the cavity size and solubility of the cyclodextrin and the relative values of these physical properties for α-, β- and γ-cyclodextrins generally reflect this [6,8,17-19] (Table **1**). Intramolecular hydrogen bonds formed between 2- and 3-hydroxyl groups on adjacent glucose units influence both the water solubility of cyclodextrins and their structural stability. Central to the usefulness of cyclodextrins in many applications is the existence of hydrophilic and hydrophobic areas of the molecules. Whereas the hydrophilic hydroxyl groups on the primary and secondary rims impart water solubility [20-22], carbon and hydrogen atoms and non-binding electron pairs of (1-4) glycosidic linkages lining the cavity create a hydrophobic environment [17,19,23,24]. It is this hydrophobic cavity that makes the cyclodextrins such attractive subjects for study and valuable molecules in product formulations particularly in the pharmaceutical, food and cosmetic industries [15,25,26].

Cyclodextrin Inclusion Complexes

The capacity of cyclodextrins to form stable inclusion complexes, also referred to as host-guest complexes, by fully or partly encapsulating other molecules within the hydrophobic cavity [2,7,10, 14, 19, 24,25,27], is widely utilized to improve solubility and stability of drugs and to reduce drug interactions, irritation, unpleasant tastes and smells [15,25,26]. In the formation of inclusion complexes in aqueous solution, energetically unfavoured water molecules in the cyclodextrin hydrophobic cavity are displaced by less polar guest molecules usually, but not always, producing a host-guest ratio of 1:1. Apart from the fact that hydrophobic guest molecules are generally bound more strongly than more polar molecules, no obvious correlation has been found between the chemical structures of encapsulated molecules and the formation and stability of resultant inclusion

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Table 1. Some Important Physical and Chemical Properties of α-, β- and γ-Cyclodextrins

Property	Cyclodextrin		
	α–	β–	γ–
Number of glucose units	6	7	8
MW (anhydrous)	973	1135	1297
pK_a (25^0C)	12.33	12.20	12.08
α_D (degrees)	+150.5	+162	+177.4
Water solubility (25^0C, $molL^{-1}$)	0.121	0.016	0.168
Height of torus (Å) approx.	7.8	7.8	7.8
Diameter of torus across secondary face (Å) approx.	14.6	15.4	17.5
Diameter of cavity across secondary face (Å) approx.	5.2	6.6	8.4

Data from [8, 17-19, 25].

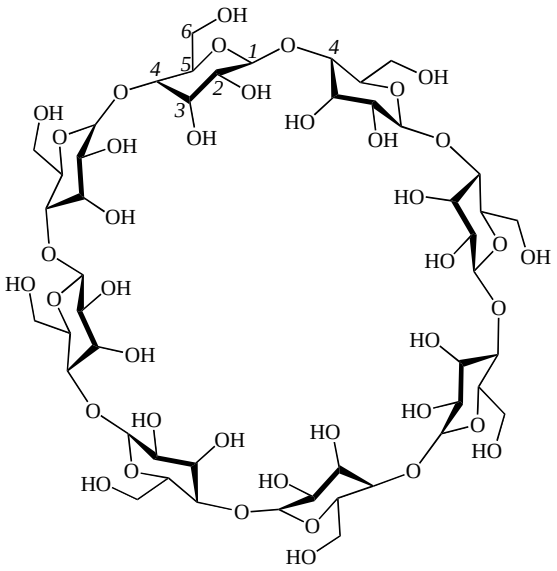

Fig. (1). Two-dimensional chemical structure of γ -cyclodextrin showing the eight glucopyranose units connected by α-1,4 bonds.

complexes [14]. The major physical forces in inclusion complex formation are hydrophobic and van der Waals interactions [28]. Of course, the size of the cyclodextrin cavity is also important; for the inclusion complex to form, the guest molecule must be able to fit into the host cavity and the size ratio of the two is not always optimum. This has led to attempts to manipulate the size of the cyclodextrin host by employing chemical modifications [8,20-22,29], for example, chemical manipulations that elongate the cavity to enable accommodation of larger guest molecules. Sugammadex, considered below, is an excellent example of the successful employment of this strategy. In this case, the addition of selected functional groups enhanced guest entrapment, accommodation, solubility and binding stability to produce a drug preparation that provided anaesthetists with a novel and useful agent to reverse neuromuscular blockade [30,31] and which may offer a new approach in the management of some cases of perioperative anaphylaxis.

NEUROMUSCULAR BLOCKING DRUGS

Neuromuscular blocking drugs (NMBDs), also called muscle relaxants, introduced into anaesthetic practice in the 1940s, relax skeletal muscle and thus facilitate endotracheal intubation and aid surgery by preventing voluntary and reflex muscle movements. The drugs are also widely used in many day care, paediatric and intensive care procedures and in critically ill patients [32]. NMBDs are divided into two categories based on their mechanism of action – depolarizing and non-depolarizing. A depolarizing NMBD (such as succinylcholine) acts as an agonist at the nicotinic acetylcholine receptor stimulating opening of the ion channels and producing muscle fasciculation. Non-depolarizing NMBDs (for example rocuronium, pancuronium and vecuronium (Fig. **2**)) act as competitive antagonists of the nicotinic receptors, blocking acetylcholine-induced activation of the ion channels thus preventing depolarization and causing muscle flaccidity [33,34]. Because of the significant number of adverse effects associated with the use of depolarizing NMBDs such as muscle pain, hyperkalaemia and cardiac arrhythmias [35], the modern non-depolarizing drugs tend to be preferred although succinylcholine is still used in selected circumstances because of its fast onset and short duration of action. Rocuronium, when compared to other non-depolarizing NMBDs, has a comparatively lower potency and, when used in large doses, has a rapid onset of action and more favourable side effects profile. Accordingly, it is a popular alternative to succinylcholine for rapid sequence inductions. To overcome postoperative neuromuscular blockade [34] caused by non-depolarizing competitive NMBDs, cholinesterase inhibitors such as neostigmine are used but these drugs have their own adverse effects including residual blockade, bronchospasm, increased airway secretion, bradycardia, nausea, vomiting and muscle cramps.

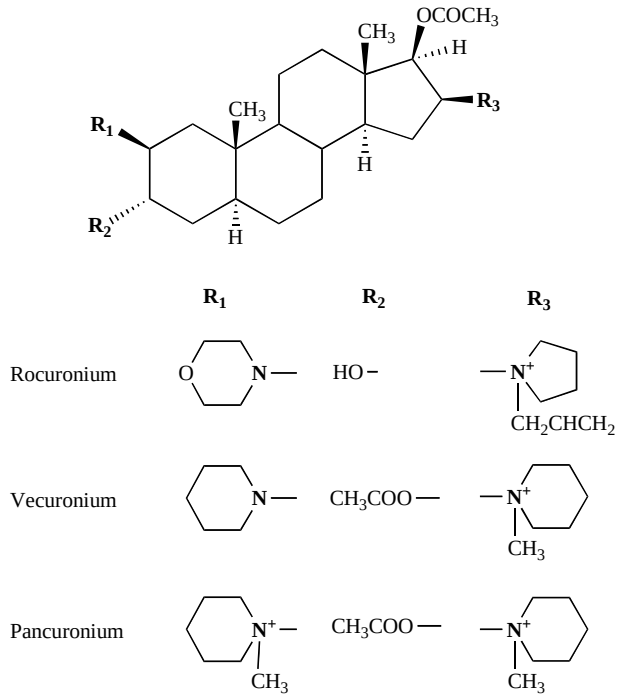

Fig. (2). Structures of the non-depolarizing and competitive aminosteroid neuromuscular blocking drugs rocuronium, vecuronium and pancuronium. Note the morpholino and pyrrolidinium groups at positions 2 and 16 respectively of rocuronium.

SUGAMMADEX

Structure of Sugammadex and Reaction with Rocuronium

Attempts to improve the solubility and decrease the pain associated with the intravenous injection of rocuronium (thought to be secondary to its acidic pH) revealed that γ-cyclodextrin was the best candidate to encapsulate the drug but binding was weak due to the host's shallow cavity which could only completely accommodate three of the six rings (the four-ring steroid nucleus plus the morpholine and pyrrolidine rings) of the rocuronium molecule (Fig. **2**). To lengthen the binding area of the cyclodextrin carrier and strengthen the binding, per-6 substitution of the primary hydroxyls with propionic acid side chains each linked *via* a thiol-ether group was undertaken [36-38] (Fig. **3**) to extend the cavity from a length of approximately 7.8Å to about 11.5Å (Fig. **4**). This is the approximate distance between the C3 carbon of ring A and C16 of ring D on the rocuronium molecule (Fig. **5**). All four rings A, B, C and D of the steroid nucleus could thus be accommodated in the extended hydrophobic cavity [36-39]. Fig. (**6**) shows three-dimensional space-filling models of sugammadex, rocuronium and the sugammadex-rocuronium inclusion complex with the pyrrolidinium quaternary ammonium group attached at position 16 of the rocuronium molecule surrounded by the attached thio(2-carboxyethyl) sodium groups visible at the primary face end of the cyclodextrin and the morpholine group containing the tertiary ring nitrogen attached at position 2 on the A ring protruding from the secondary face.

This chemically manipulated γ -cyclodextrin (6^{A-H}-oktakas-S(2-carboxyethyl)-6^{A-H}-octathio-γ-cyclodextrin octasodium salt), given the generic name sugammadex and synthesized as a selective binding agent for rocuronium, shows a 1:1 binding ratio with the drug and binds to it with an extremely high affinity (K_a 1.8 x 10^7 M^{-1}), a very low dissociation constant and with specificity for the aminosteroid NMBDs [36,37,39,40]. This binding affinity is approximately 1000 times higher than the figure obtained for the rocuronium- unmodified γ-cyclodextrin complex and two

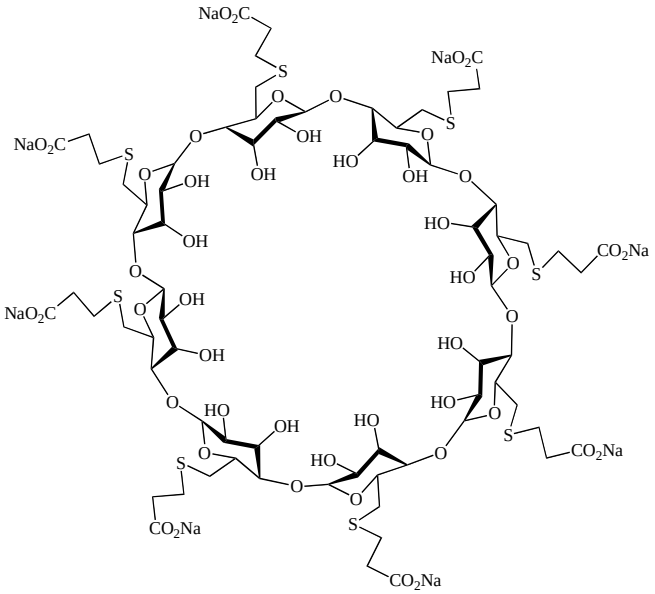

Fig. (3). Two-dimensional structure of sugammadex, 6-perdeoxy-6-per(2-carboxyethyl)thio-γ-cyclodextrin sodium salt showing the thio(2-carboxyethyl) sodium group linked at position 6 of each of the eight glucopyranose units. Refer to Fig. (**1**) for the structure of unmodified γ-cyclodextrin.

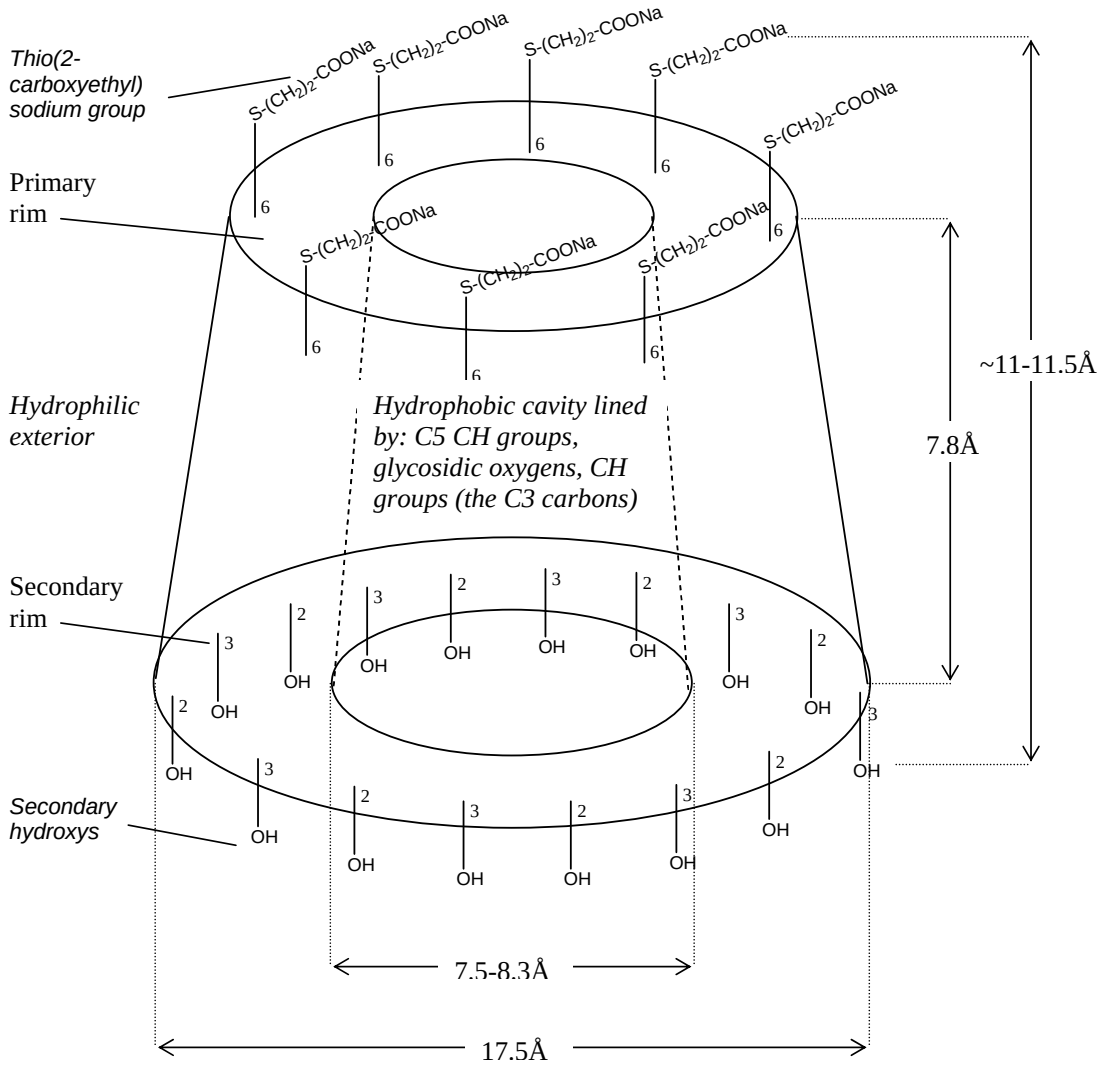

Fig. (4). Diagrammatic representation of the toroidal shape of sugammadex showing the attachment of the eight thio(2-carboxyethyl)sodium groups at the primary ring end and indicating the ~ 11–11.5Å distance of the extended cavity (see Fig. **5**).

and a half times the vecuronium-sugammadex figure. Pancuronium is less strongly bound again. Specificity of sugammadex binding was demonstrated by results showing that the binding affinity of rocuronium was 120-700 times greater than the binding observed with a range of more than 40 drugs given to anaesthetized patients including other steroids, induction agents, opioids, antibiotics, bronchodilators and cardiovascular drugs [41]. Although steroids such as cortisone, hydrocortisone and aldosterone complex with sugammadex, the binding affinities are more than 120 fold less than that of rocuronium, indicating, according to Zhang [41], that the interaction of the positively charged ammonium ion of rocuronium with carboxyl groups on the extended cavity accounts for its higher binding affinity.

Use of Sugammadex to Reverse Neuromuscular Blockade

Given that neostigmine is still the most common anticholinesterase drug used, that it has some undesirable effects and that no significant progress in the area of neuromuscular antagonism had been made since the early 2000s [42], there remained a clinical need to develop an agent(s) that reversed neuromuscular blockade without inhibiting acetylcholinesterase. Given the current popularity of rocuronium as a NMBD and the property of sugammadex to specifically sequester the drug as an inclusion complex with a high binding affinity, application of the modified cyclodextrin for the reversal of rocuronium-induced block by removal of the drug from the neuromuscular junction soon followed [30,31,42]. Sugammadex facilitates reversal of neuromuscular blockade by rapidly binding to and effectively removing rocuronium from the plasma thus creating a concentration gradient that moves the drug from the neuromuscular junction to the plasma [43-47] and ultimately to the kidneys where the intact complex is excreted in the urine [46, 48]. Vecuronium and pancuronium are also bound but less strongly than rocuronium and reversal of neuromuscular blockade with other NMBDs such

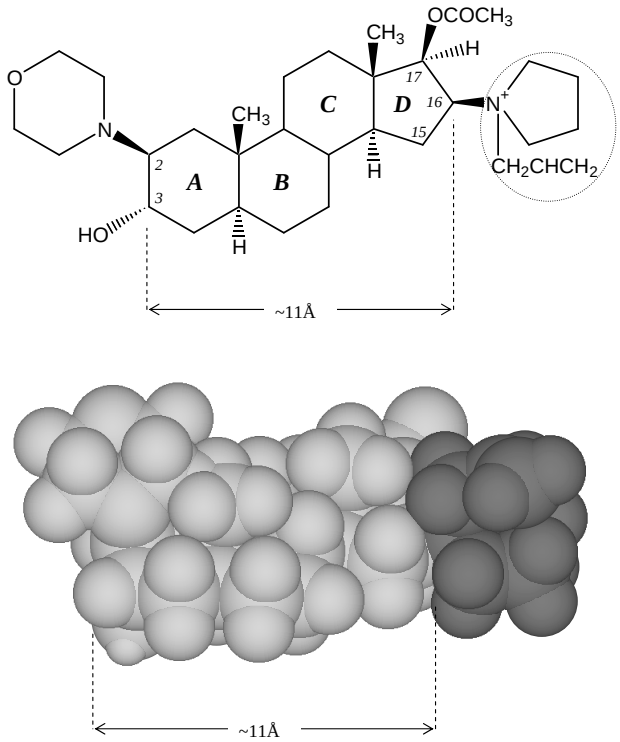

Fig. (5). Two-dimensional chemical structure and space filling CPK molecular model of rocuronium showing the approximate 11Å distance between the C-3 atom on ring A of the steroid nucleus and the C-16 atom on ring D. The propenyl pyrrolidinium quaternary ammonium group is circled on the two-dimensional structure and dark shaded on the CPK model. See Figs. (**4** and **6**) for the significance of the ~11Å distance.

as succinylcholine and the benzylisoquinoliniums does not occur as these molecules do not form inclusion complexes with sugammadex [49,50].

PERIOPERATIVE ANAPHYLAXIS TO NEUROMUSCULAR BLOCKING DRUGS

Mechanisms Underlying Anaphylaxis to Neuromuscular Blocking Drugs

NMBDs are the most common cause of anaphylaxis during the perioperative period far exceeding other anaphylaxis-provoking agents such as colloids, latex, hypnotics, antibiotics and opioids [51-56]. The dominant allergenic determinant on all NMBDs is a substituted (quaternary or tertiary) ammonium ion, sometimes with neighbouring structures [57-62] and since all NMBDs contain substituted ammonium groups, there is immunological cross-reactivity both clinically in the patient and *in vitro* in IgE antibody-binding studies [58,60-67]. Since NMBD-carrier antigenic complexes have not yet been shown to form and allergically sensitize subjects, the origin of the allergenic stimulus for the anaphylaxis in NMBD-sensitive patients remains a mystery [58,62]. All NMBDs contain at least two substituted ammonium ions separated by a distance of at least 1 nm [33] and this has lead to the suggestion that the ammonium groups initiate the release of mediators of anaphylaxis by each interacting with, and bridging, mast cell- and basophil-bound NMBD-reactive complementary IgE antibodies [58,60,62]. Different possible types of bridging cell-bound antibodies and triggers for mediator release by drugs and simple chemicals have also been discussed by Schneider *et al*. [68,69].

Sugammadex and the Apparent Reversal of Rocuronium-Induced Anaphylaxis

Coincident with the popularity of rocuronium for neuromuscular blockade has been an increase in reports of anaphylaxis to the drug and although some believe that the increase is due to increased allergenic potential of the drug [53,66,70-72], others are of the opinion that the findings are merely a reflection of market share [73-75]. In drawing attention to the obvious necessity of promptly eliminating ongoing patient exposure to any agent provoking an anaphylactic reaction and the difficulties associated with the immediate and complete removal of an antigen after intravenous administration, Jones and Turkstra [76] suggested that sugammadex may offer a novel treatment strategy in the management of patients experiencing an anaphylactic reaction to rocuronium. Mention was also made of the possibility that the allergenic ammonium group of rocuronium in the sugammadex inclusion complex might still be accessible for binding to complementary IgE antibodies. If this were so, the treatment strategy would probably fail. In emphasizing the uncertainties associated with the proposal, Harper [77] concluded that, "There is clearly much to be learned before sugammadex can be used in the management of rocuronium-induced anaphylaxis."

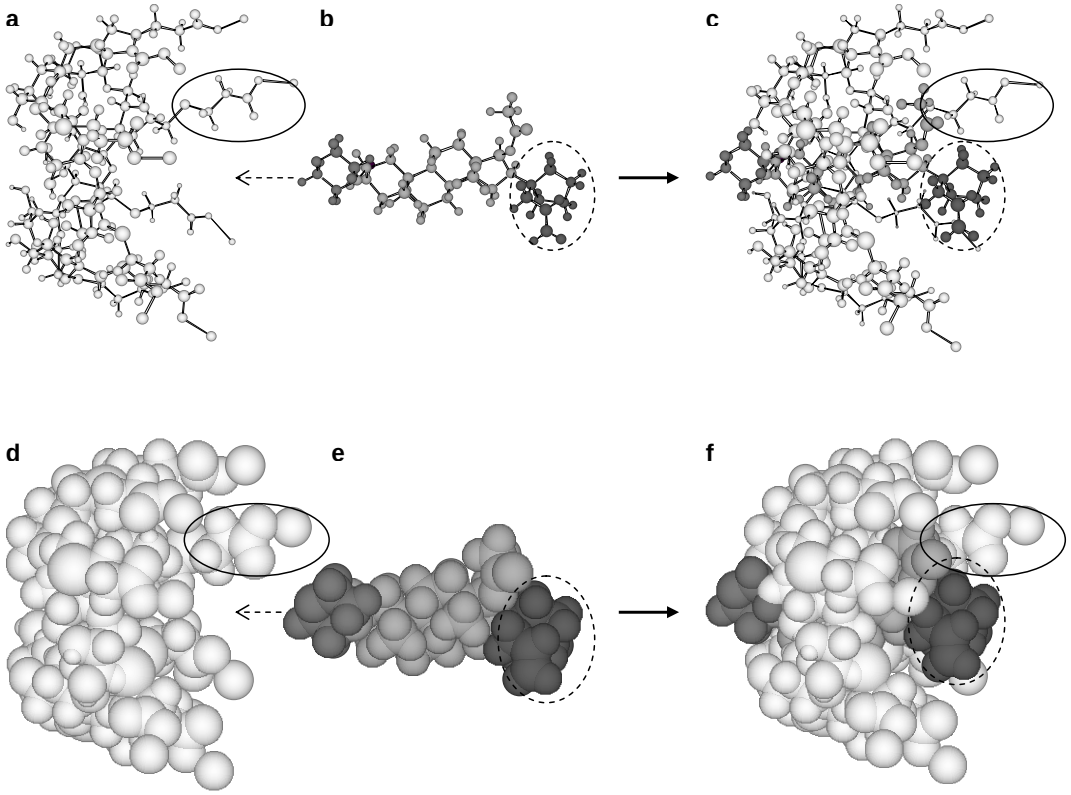

Fig. (6). Space filling ball-and-stick (**a**,**b**,**c**) and CPK (**d**,**e**,**f**) three-dimensional molecular models illustrating the encapsulation of the rocuronium molecule (**b** and **e**) by sugammadex (**a** and **d**) to form the rocuronium-sugammadex inclusion complex (**c** and **f**). The pyrrolidinium quaternary ammonium group (circled) and the tertiary ammonium group, part of the morpholine ring, are shown in dark shading at the right and left hand ends respectively of the rocuronium molecule. In the inclusion complex, the quaternary ammonium group attached to ring D of rocuronium (see Fig. **5**) is visible at the primary rim end (right hand side) surrounded by thio(2-carboxyethyl) sodium groups (**c**,**f**). One of the eight thio(2-carboxyethyl) sodium groups of sugammadex is ringed. The morpholine group containing the tertiary ring nitrogen and attached at position 2 of the rocuronium molecule protrudes from the complex at the secondary rim end (left hand side). The hydroxyl group at position 3 is behind the morpholine ring in the selected view. All four rings A-D of the rocuronium steroid nucleus and the ~11Å C3 -- C16 length are within the sugammadex extended cavity.

Clinical findings relevant to the above comments were soon obtained and provided in the form of some recently published case reports. In the first case, a bolus of sugammadex (6.5 mg/kg) was found to apparently quickly reverse a difficult to manage rocuronium-induced anaphylactic reaction involving cardiovascular collapse [78]. At least two other similar cases have been described [79,80] although a recent similar case with similar onset and recovery time-frames was resolved successfully without the use of sugammadex [81]. It is clearly not possible to conduct a clinical trial to examine the question of the efficacy of sugammadex in the treatment of rocuronium- (or vecuroniun-) induced anaphylaxis. The obvious ethical considerations for such a trial and infrequent occurrence of the anaphylactic reactions means that further examination of this question can only be carried out by accumulation and assessment of intermittent reports of isolated cases, appropriate skin test investigations and/or by the execution of appropriate carefully planned *in vitro* studies.

Some investigations prompted by the questions raised by Jones and Turkstra have already been presented. Utilizing the basophil activation test together with flow cytometric analysis to detect the cell surface marker CD63, Leysen *et al*. [82] challenged blood samples from skin test-positive rocuronium-allergic patients with preincubated mixtures of rocuronium and sugammadex and found that sugammadex inhibited rocuronium-induced basophil activation. On-going basophil activation induced by rocuronium could not be prevented by sugammadex but relevant to this conclusion is the relationship between the presence of the CD63 marker already expressed on the basophil surface and a continuing allergic reaction. In other words, is the presence of CD63 always an indicator of active and *continuing* release of

inflammatory and allergic mediators? The finding that preincubation of rocuronium with sugammadex prevented basophil activation and CD63 expression, suggests that allergenic structures on the bound and encapsulated drug were not available to react with cell bound anti-rocuronium IgE antibody molecules. As yet, however, no direct quantitative immunochemical studies of antibody recognition and binding to the rocuronium-sugammadex complex, or any other drug-cyclodextrin complex, appear to have been carried out. One should also keep in mind that NMBD-reactive IgE antibodies have been shown to react with tertiary as well as quaternary ammonium ions [57,58,60] and rocuronium contains one of each of these groups at opposite ends of the molecule (Fig. **2**). The implications of this are discussed below.

Whether the allergenic structures on rocuronium are inaccessible, fully accessible, or even partly accessible to IgE binding when in the inclusion complex form has still not been definitively resolved and, in the light of the clinical findings [78-81] outlined above, it remains of interest to firmly establish whether or not sugammadex can sequester rocuronium from cell-bound rocuronium - IgE antibody complexes. Clearly, some suitable laboratory investigative procedures are needed.

EXPERIMENTAL STRATEGIES TO INVESTIGATE BINDING RELATIONSHIPS BETWEEN FREE DRUG, CYCLODEXTRIN CARRIER, CYCLODEXTRIN-DRUG INCLUSION COMPLEX AND IGE ANTIBODIES

While both *in vitro* binding affinity and specificity studies on the binding of aminosteroid NMBDs to sugammadex and *in vivo* results showing reversal of neuromuscular blockade and apparent reversal of anaphylaxis after intravenous sugammadex suggest that the cyclodextrin might be a valuable new treatment for rocuronium- (and perhaps vecuronium-) induced anaphylaxis, two important questions related to IgE antibody binding remain. These questions will now be discussed together with experimental strategies designed to provide clear and easy to interpret answers.

Are the Drug Allergenic Structures on the Rocuronium-Sugammadex Complex still Accessible to IgE Binding?

By extending the γ-cyclodextrin cavity by three carbon atoms to accommodate all four hydrophobic rings of the rocuronium gonane nucleus, the depth of the cavity, as measured from the distances between the furthest hydroxyl oxygen atom on the secondary face and the most extended carboxyl oxygen atom attached at the primary face end, is up to 11.5 Å (Fig. **4**), approximately the same length as the distance between the C3 carbon on the A ring of rocuronium and the C16 carbon on the D ring (Fig. **5**). X-Ray crystallography [36,37] revealed rocuronium as an inclusion within the cyclodextrin cavity with the 2-morpholino and 3-hydroxy groups attached at ring A protruding from the cavity at the secondary hydroxyl end. The four rings A – D making up the steroid nucleus are essentially within the enlarged cavity while at the primary end, the acetoxy and propenyl pyrrolidinium quaternary ammonium groups attached to ring D at positions 17 and 16 respectively are loosely surrounded by the (2-carboxyethyl)thio groups. Interestingly, and despite the alleged interaction between the positively charged nitrogen and the carboxyl groups, no direct interaction between these groups was observed [36]. It seems possible, therefore, that at each end of the encapsulated rocuronium molecule, a substituted ammonium group is potentially exposed to recognition by, and reaction with, rocuronium-reactive IgE antibodies. Tertiary and quaternary ammonium groups may react with NMBD-reactive IgE antibodies [57,58,83] so both the tertiary ring nitrogen of the morpholino group and the pyrrolidinium group may serve as recognition sites for IgE in the sera of rocuronium-allergic subjects. If sugammadex is to be further employed to assist with the management of rocuronium-induced anaphylaxis, it is important to know whether or not encapsulated rocuronium in inclusion complex form is allergenically masked or if its quaternary and/or tertiary ammonium groups remain accessible for interaction with rocuronium-reactive IgE antibodies.

Laboratory experiments based on classical antibody direct-binding and quantitative hapten inhibition methods, and designed to directly answer the question of whether or not the allergenic structures on the sugammadex-rocuronium inclusion complex are accessible and react with IgE, are summarized diagrammatically in Fig. (**7**). IgE antibodies in the serum of a subject allergic to a drug can be detected with a solid phase form of the drug, usually the drug itself or a structurally related compound [58,59,83-86], chemically coupled to an insoluble support such as cellulose, Sepharose, polystyrene, etc (Fig. **7a**). Antibody bound to the drug solid phase is detected with a second antibody, a labeled anti-human IgE. To confirm antibody specificity for the drug, serum is preincubated with the free drug (step 1; Fig. (**7b**)) before addition of the drug solid support (step 2; Fig. (**7b**)). Antibody combining sites that remain unblocked (uninhibited) by free drug are free to react with the immobilized drug on the solid phase so specific antibody recognition of the drug is reflected in significantly reduced antibody binding to the immobilized drug compared to the binding observed in the direct binding (non-inhibited) assay (Fig. **7a**). For example, antibody reactivity of rocuronium-(or vecuronium-) sugammadex inclusion complex can be investigated by using these direct binding and inhibition procedures. Given that sugammadex and rocuronium bind in a 1:1 ratio, quantities reflecting the stoichiometric ratio of the compounds are mixed (step 1; Fig. (**7c**)) to produce the guest-host inclusion complex. The mixture is dialysed to remove any unbound drug (MW rocuronium bromide 609.7) but retain sugammadex (MW 2178) and then serum containing IgE antibodies to rocuronium (step 2; Fig. (**7c**)), followed by rocuronium-solid phase, are added (step 3; Fig. (**7c**)). After incubation and centrifugation and washing (to remove excess IgE antibodies), detection of IgE antibodies (by addition of labeled anti-human IgE) on the rocuronium-solid phase, that is, in the residue, indicates that the added IgE antibodies to rocuronium are unable to bind to the encapsulated rocuronium molecules and were thus free to react with the immobilized drug on the solid phase (Fig. (**7d**)). On the other hand, absence of, or little, bound IgE antibodies on the drug-solid phase in the residue but binding

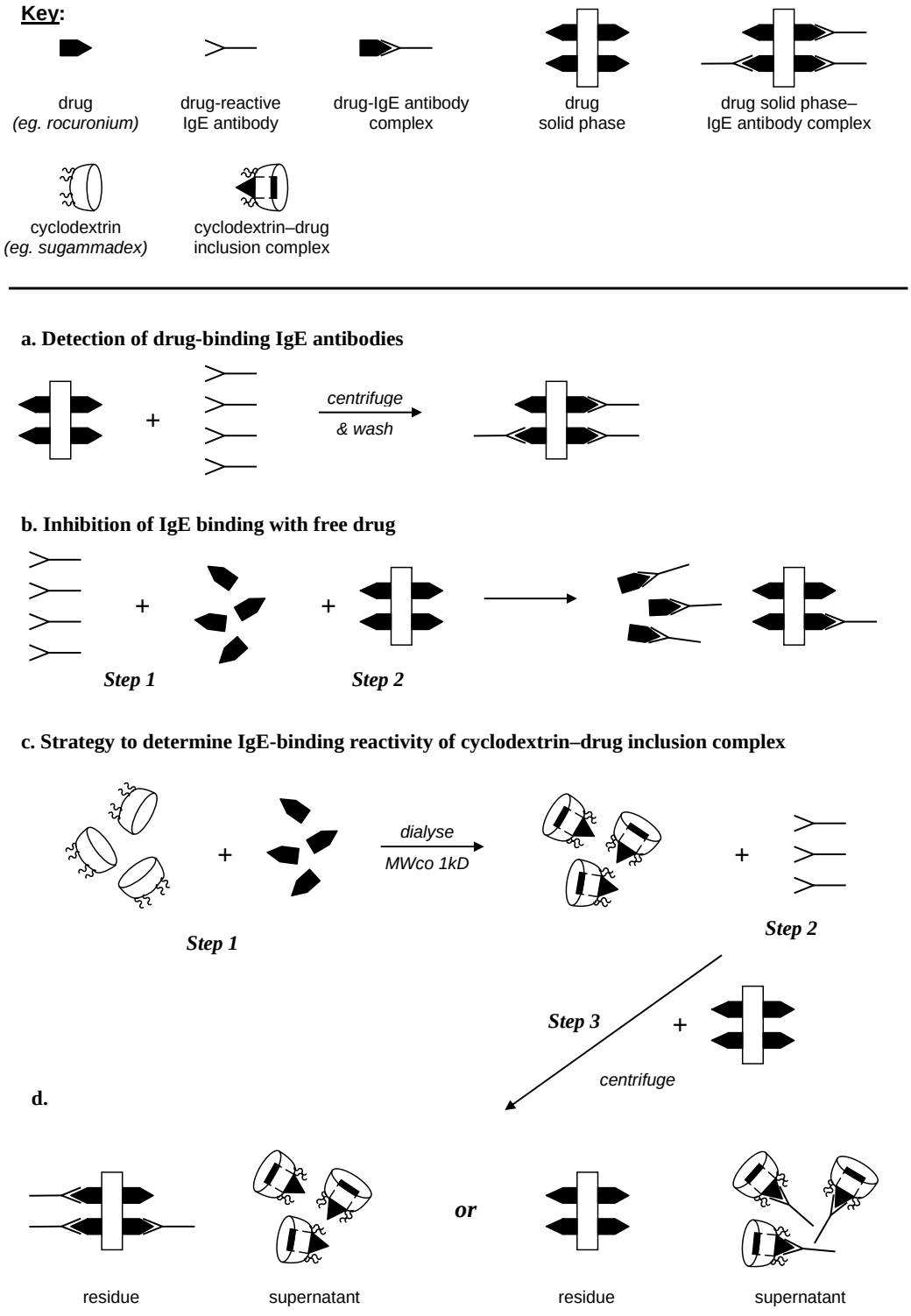

Fig. (7). Strategies and experimental procedures to determine if the rocuronium allergenic structures in the sugammadex-rocuronium inclusion complex are accessible for binding with rocuronium-reactive IgE antibodies. Bound IgE antibodies are detected with a labeled anti-human IgE. (Note: The experimental protocols outlined here are purely diagrammatic and designed for schematic simplicity and brevity. Reactants are shown as simple shapes and no attempt was made to accurately reflect detail, for example, the relative size differences of the reactants and the multi-chain structure of antibodies).

in the supernatant, indicates that the added rocuronium-reactive IgE antibodies in serum gained access, and bound to the rocuronium in the sugammadex inclusion complex (Fig. **7d**). The *in vivo* reflection of such a result would be that the drug bound in the inclusion complex was still available for binding to the patient's IgE antibodies and thus sugammadex would be unlikely to inhibit an ongoing allergic reaction to the drug. A more direct, but laborious, procedure to demonstrate IgE antibody binding to the encapsulated drug or the drug-solid phase involves the employment of purified and radiolabeled drug-reactive IgE antibodies isolated from allergic patient's serum at step 2 (Fig. **7c**) without the addition of the drug solid phase.

Can Sugammadex Compete with IgE Antibodies for Free Rocuronium or Sequester the Bound Drug from IgE-Rocuronium Complexes?

Experimental procedures to investigate the question of competition for free drug between antibodies and sugammadex are summarized in Fig. (**8a,b**). Radiolabeled rocuronum (tritium or carbon) is added to a mixture of sugammadex and patient's serum containing rocuronium-reactive IgE antibodies and, after incubation, free rocuronium is removed by dialysis. A second round of dialysis is then undertaken using a membrane to separate sugammadex and antibody and the dialysate and retentate are retained and counted Fig. (**8a**). Radioactivity in the retentate (high MW fraction containing IgE antibodies) but not in the dialysate (low MW fraction containing sugammadex) indicates that the labeled drug is bound to the antibody fraction and not to sugammadex molecules. Counts in the dialysate but not in the retentate show the reverse suggesting that, *in vivo*, sugammadex might show greater affinity for rocuronium and outcompete IgE molecules for the drug.

An experimental strategy to examine competition for rocuronium between sugammadex and immobilized rocuronium-reactive, instead of free, IgE antibodies, is summarized in Fig. (**8b**). The general experimental strategy remains the same but the second dialysis step is replaced by a centrifugation and washing step and the IgE antibody solid phase and sugammadex reactants end up in the residue and supernatant (plus washings) fractions respectively. As above, radioactivity in the residue or the supernatant would indicate drug bound to antibody or to sugammadex respectively.

It is also important to obtain an answer to the question of whether or not the uncomplexed cyclodextrin can remove the drug from the IgE combining sites and form an inclusion complex with it. This can be investigated in the following way. After forming IgE antibody-rocuronium complexes by adding radiolabeled rocuronium to serum containing anti-rocuronium IgE antibodies and dialysis to remove unbound labeled drug, sugammadex is added and the mixture is incubated (Fig. **8c**). At various time intervals extending from a few minutes to hours, samples are taken, dialysed using a membrane to remove sugammadex but retain antibody molecules and both dialysate and retentate are counted. Radioactivity in the retentate (high MW fraction) and its absence from the dialysate (low MW fraction) indicates that the labeled drug remains bound to the antibody fraction and that the sugammadex molecules did not sequester the rocuronium from the IgE combining sites. Radioactive counts in the dialysate and their absence from the retentate, however, shows that the sugammadex 'clawed back' the rocuronium from the IgE-drug complex. The latter finding would suggest that a transfer of rocuronium might occur *in vivo* in patients experiencing anaphylaxis to the NMBD.

Again, to examine competition using immobilized antibody, IgE antibodies in solid phase form are substituted for the IgE antibodies free in serum. After incubating together the immobilized antibodies and labeled rocuronium, centrifugation and washing are necessary to remove any unbound free drug before finally adding sugammadex and centrifuging and washing again (Fig. **8d**). Interpretation of findings is as for Fig. (**8b**).

DISCUSSION

Sugammadex was developed to reverse rocuronium-induced neuromuscular blockade and although its high binding affinity, low dissociation constant, restricted specificity and efficacy in encapsulating the NMBD and rapidly restoring normal muscle function potentially exceeded expectations, the reported rapid alleviation of anaphylactic symptoms [78-80] is somewhat surprising. To so quickly alleviate allergic symptoms suggests that the modified cyclodextrin not only removes the free drug in the body but also, apparently, sequesters the antibody-bound rocuronium cross-linking the complementary cell-bound IgE antibodies. If it were found that sugammadex encapsulates only the free rocuronium, then it would seem that abrupt interruption to the on-going exposure to the provoking antigen is more important in shutting down the allergic reaction than removing the NMBD from the drug-IgE cell-bound complexes. This appears to be unlikely since it would further suggest that rapidly turning off the on-going supply of antigen also rapidly interrupts the allergic cascade with its release of detrimental mediators [87,88] even though the antibody-bound antigen cross-linking mast cells and basophils remained undisturbed. This scenario does not fit with our current understanding of allergen-induced release of the mediators of anaphylaxis [87,88]. Average association constants for the interaction of allergen-specific IgE antibodies with their complementary allergens are high, often in the range 10^{10} - 10^{11} M^{-1} and often higher than the corresponding IgG responses [89,90]. Although this is significantly higher than the association constant (K_a) for the sugammadex–rocuronium interaction (1.8×10^7 M^{-1}), there is no available data on the strength of binding and stability of IgE–NMBD complexes. Since it seems likely that many (if not most or all), patients who experience NMBD-induced anaphylaxis are not allergically sensitized by a NMBD [58,62], both the affinity and avidity of the IgE antibody–NMBD interaction may be considerably lower than in most reactions where the IgE antibodies are formed in direct response to the complementary allergen where a better 'fit' is generally the result. In such a situation, the relative affinities and avidities of the IgE–rocuronium and sugammadex–rocuronium interactions might be much closer or even higher in the latter case and that might lead to a concentration gradient with transfer of the drug from the combining sites of the cell-bound antibody to the cavity of the cyclodextrin.

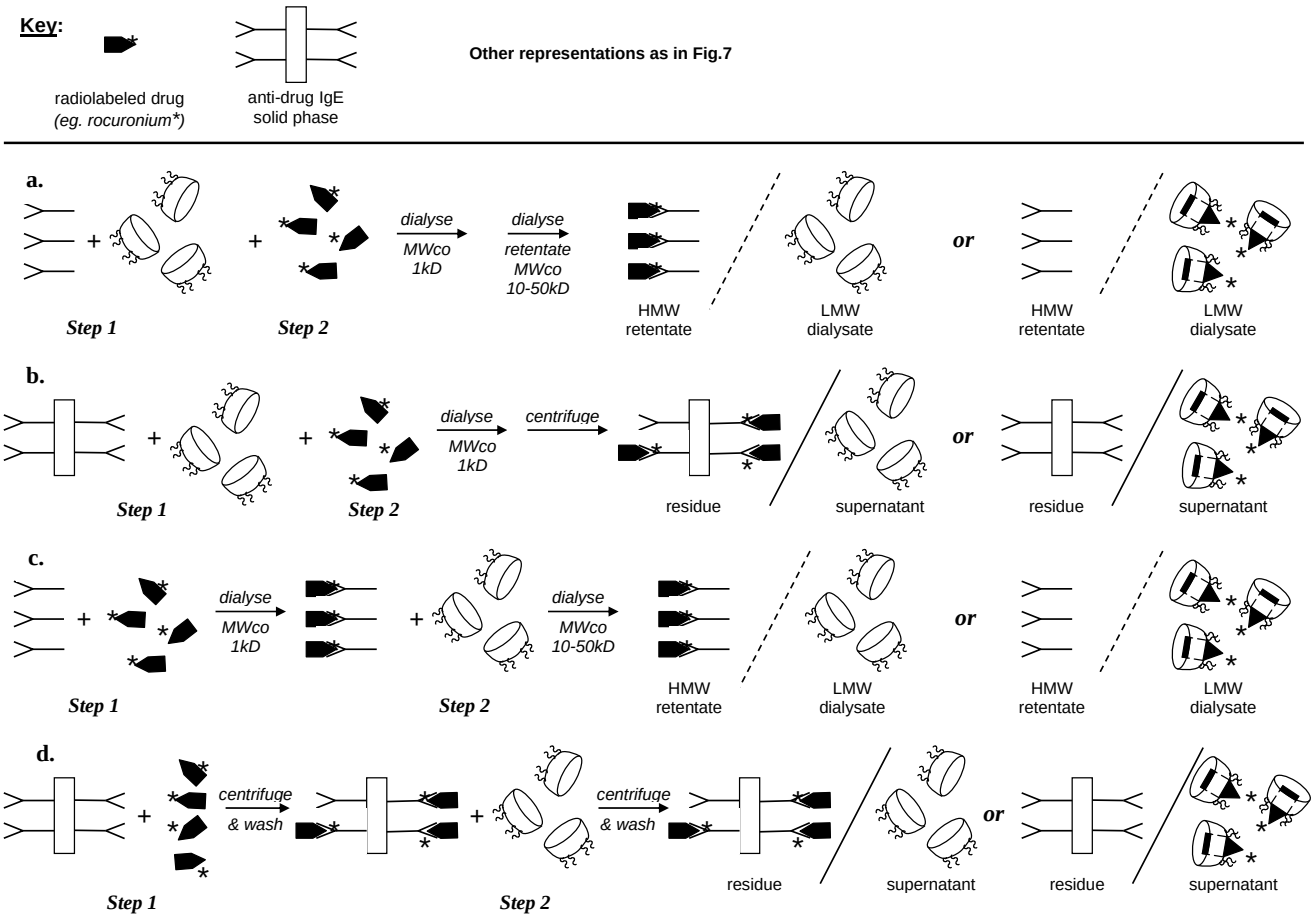

Fig. (8). Competition between sugammadex and IgE antibodies for rocuronium. Strategies and experimental procedures to determine if: Sugammadex can compete for rocuronium with (**a**) free and (**b**) immobilized rocuronium-reactive IgE antibodies; Sugammadex can remove bound rocuronium from rocuronium-IgE antibody (**c**) soluble and (**d**) immobilized complexes. Bound IgE antibodies are detected with a labeled anti-human IgE. (See also note in parentheses, Fig. (**7**)).

This point can be investigated, in the first instance by measuring the average association constants for the interactions of a number of different rocuronium-reactive IgE antibodies with the drug. Affinities for antibodies reacting with the same hapten may differ by a factor of 10^3 to 10^5 [91] so it is possible that NMBD-reactive IgE antibodies may show similar heterogeneity, resulting in sugammadex alleviating an anaphylactic reaction provoked by rocuronium in some patients but not in others. It will be interesting to determine therefore whether or not there is a correlation between the K_a of sugammadex and its efficacy in reversing on-going anaphylactic reactions to rocuronium or vecuronium and the average association constants of rocuronium- or vecuronium-reactive IgE antibodies in the sera of allergic patients. Mitigation of anaphylaxis should result in patients when the affinity of sugammadex for rocuronium is higher than the affinity of IgE antibodies for rocuronium. In the reverse situation, anaphylaxis would proceed.

If experimental findings demonstrate that rocuronium-reactive IgE antibodies bind to the drug in the sugammadex inclusion complex, it will be necessary, and of great interest, to identify the complementary binding structures on the rocuronium molecule. Both the tertiary ammonium nitrogen, part of the morpholino group, and the quaternary pyrrolidinium group, each at opposite ends of the molecule, are potentially reactive with rocuronium-reactive IgE antibodies [57,58,60,62] but access to both structures appears to be at least partly restricted by surrounding and nearby structures of the cyclodextrin host. Reactivity with either, or both, substituted ammonium ions would provide valuable information on the extent and completeness of enclosure of the guest molecule at the primary and secondary rim ends of the sugammadex host and be instructive for future efforts to lock away drugs or other reactive molecules from recognition or interaction.

CONFLICT OF INTEREST

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