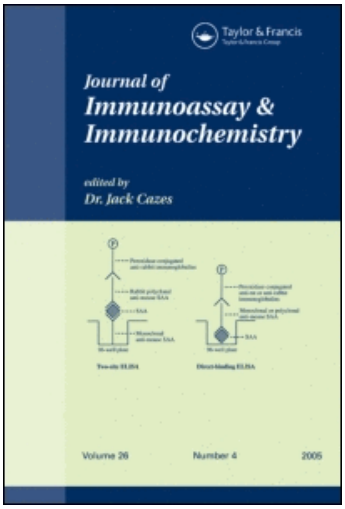

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STUDIES ON THE MECHANISM OF MULTIPLE DRUG ALLERGIES. STRUCTURAL BASIS OF DRUG RECOGNITION

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STUDIES ON THE MECHANISM OF MULTIPLE DRUG ALLERGIES. STRUCTURAL BASIS OF DRUG RECOGNITION

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ABSTRACT

The multiple drug allergy syndrome, that is, allergic recognition of a variety of drugs that may be both pharmacologically and structurally different, has been little studied and, consequently, the underlying mechanism(s) is unknown. The molecular basis of drug recognition by IgE antibodies found in the sera of subjects exhibiting multiple allergic drug sensitivities was studied by direct binding and quantitative hapten inhibition assays in experiments employing a wide range of carefully selected drugs and other chemicals.

Drug recognition was shown to be related to the presence of tertiary and quaternary mono-, di- and trialkyl amino groups,

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but only if the alkyl groups were 'small' viz., methyl or, perhaps, ethyl. Primary, secondary, and tertiary (with R = 'large' alkyl) groups showed no direct antibody binding or antibody inhibitory activities. Near-neighbour effects of amide and hydroxyl groups appeared to promote weaker antigenic recognition.

Results indicate that the antibody recognition and clinical drug allergy spectra of at least some subjects with multiple drug allergies are due to wide ranging immunological cross-reactivities with drugs containing tertiary amino and quaternary ammonium groups which are present in many different pharmacologically active agents. Separate populations of antibodies to other non-cross reacting drugs, for example, β-lactam antibiotics, may also be present in the sera of such subjects.

INTRODUCTION

It seems that some individuals are at an increased risk of reacting to a variety of drugs that are both pharmacologically and structurally different. For example, some studies have shown that patients with a history of allergy to penicillin or other antimicrobial drugs are more likely to have allergic reactions to other drugs,(1-7) but this was not supported in a recent examination of penicillin-allergic subjects showing multiple drug reactions.(8) The mechanism(s) underlying the so-called multiple drug allergy syndrome remains unknown, although the weak suppression of immune responses to hapten has been suggested to account for the diversity of haptens recognized and reaction patterns seen.(2)

In our studies, some patients displayed allergic sensitizations to a variety of drugs that, on superficial inspection, appeared to be immunochemically and, sometimes, pharmacologically distinct. In these patients, *in vitro* tests for drug-reactive IgE antibodies showed that morphine was one of the most commonly and strongly recognized agents. In an attempt to distinguish whether such allergic sensitivities are due to separate sensitizations by individual drugs or recognition of a small, common feature on the different drugs, the drug recognition spectra of IgE antibodies from patients were studied qualitatively and quantitatively using hapten inhibition methods.

EXPERIMENTAL

Drugs and Chemicals

Compounds used, and the suppliers, were: trimethylamine hydrochoride, triethylamine, ethanolamine, choline chloride, naloxone hydrochloride, benzylpenicillin sodium, ampicillin sodium, cephalothin sodium, cephalexin, cefaclor, pipemidic acid, flumequine, cinoxacin, ofloxacin, human serum albumin (HSA) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) [Sigma Chemical Co., St. Louis, MO, USA].

Methylamine hydrochloride, ethylamine hydrochloride, dimethylamine hydrochloride, diethylamine hydrochloride, dipropylamine, dibutylamine, dimethylethylamine, diethylmethylamine, tripropylamine, tributylamine, ethylethanolamine, propylethanolamine, dimethylethanolamine, diethylethanolamine and dibutylethanolamine [Aldrich Chemical Co., Milwaukee, WI, USA].

Methylethanolamine and butylethanolamine [Fluka Chemika-BioChemika, Buchs, Switzerland]. Thiopentone sodium [Abbott Australasia Pty. Ltd., Sydney, Australia]. Naltrexone hydrochloride [Commonwealth Serum Laboratories, Vic, Australia]. Fentanyl citrate [Janssen Pharmaceutica Pty. Ltd., NSW, Australia]. Alcuronium chloride, sulfamethoxazone and trimethoprim [Roche Products Pty. Ltd., NSW, Australia]. Bupivacaine hydrochloride, lignocaine hydrochloride, pancuronium bromide and suxamethonium chloride [Astra Pharmaceuticals Pty. Ltd., NSW, Australia].

Procaine hydrochloride, atracurium besylate and morphine sulfate [David Bull Laboratories, Vic, Australia]. Promethazine hydrochloride and gallamine triethiodide [May & Baker Australia Pty. Ltd., Vic, Australia]. Rocuronium bromide and vecuronium bromide [Organon Teknika Pty. Ltd., Sydney Australia]. Methadone hydrochloride, nalorphine hydrochloride and *d*-tubocurarine chloride [Wellcome Australia Ltd. Pty., NSW, Australia].

Tetracycline hydrochloride and minocycline hydrochloride [Cyanamid Australia Pty. Ltd., NSW, Australia]. Codeine phosphate and doxycycline hydrochloride [the Pharmacy, Royal North Shore Hospital, Sydney, Australia]. Ciprofloxacin hydrochloride [Pentex-Miles Inc., Kankakee, Illinois, USA]. Pefloxacin [Mediolanum Farmaceutici S.p.A., Milan, Italy]. Epoxy-activated (EA) Sepharose 6B [Pharmacia Biotech AB, Uppsala, Sweden] and ^{125}I-anti-human IgE [Bioclone, NSW, Australia].

Subjects and Sera

Studies were undertaken on sera from 7 patients who showed marked clinical reaction to one or more drugs. Reactions ranged from rashes, flushing, sneezing, urticaria, and swelling of lips to anaphylaxis. Clinical details of these subjects are summarized in Table 1. Tests for serum IgE antibodies to the suspected provoking drugs and to variety of other drugs, often unrelated structurally and pharmacologically, were found to be positive. Control sera were obtained from 'normal' non-allergic adult subjects, from patients clinically diagnosed as allergic (rhinitis and/or asthma) to house dust mite or pollens and from subjects with a high total IgE level but non-allergic to drugs. Cord serum was provided by the Department of Obstetrics, Royal North Shore Hospital, Sydney, and used as an IgE-'free' control. All sera were stored at –20°C.

Preparation of Drug–Sepharose Covalent Complexes

The preparation of Sepharose solid phases complexes of alcuronium,(9) *d*-tubocurarine,(10) choline, triethylcholine and ethanolamine, (11,12) morphine,(13) vecuronium,(14) thiopentone,(15) trimethoprim, (16) and cephalothin(17) have been described elsewhere. The general method used for producing these drug-Sepharose complexes is similar to the procedure applied for new preparations described below, with the exception that Sepharose 4B activated with divinyl sulphone at pH 10 was employed with *d*-tubocurarine.

For the development of drug-Sepharose covalent complexes via bisoxirane coupling, optimum preparations were selected after investigating IgE antibody binding of complexes prepared at different pHs. Conditions selected for the preparation of solid phase complexes of dimethylethanolamine, tetracycline, cephalexin, cefaclor, pipemidic acid and ofloxacin were as follows: Drug (120 mg, but 1 mL for dimethylethanolamine) was dissolved in suitable diluent (about 10–20 mL of distilled water for dimethylethanolamine and tetracycline, 0.1 M NaOH for cephalexin and cefaclor, and sodium carbonate-sodium bicarbonate buffer pH 10.8 for quinolones) and then mixed with EA Sepharose 6B (1 g). The pH was adjusted to 12.0 with 2.5 M NaOH and the mixture gently shaken at 25°C for 24 h. After washing with water, 0.1 M borate buffer pH 8 and 0.1 M acetate buffer pH 4, the remaining free activated groups on the gel were blocked by incubation with 1 M ethanolamine pH 9 at room temperature overnight. The gel was washed again as above, finishing with a final wash of distilled water and then

Table 1. Clinical Details of Subjects with a Clear History of Multiple Drug Reactivity*

Subject	Sex & Age	Symptoms/Clinical Notes & Details of Most Recent Reactions	Drug(s) Used at Time of Last Reaction	Tests Requested
Car	, **F,**	**Swelling of lips with doxycycline (May 95)**	**Doxycycline**	**Doxycyline,** penicillins, cephalosporin
Ley	F, 58	Multiple previous anaesthesias. On one occasion low blood pressure on induction, thought to be due to meperidine. On this occasion, following induction with fentanyl, propofol and scoline, blood pressure undetectable, carotid pulse palpable, erythematous rash (arms, neck, chest). Suspected reaction to scoline	Scoline and the other drugs used	Anaesthetic agents
Dow	M, 16	Anaphylaxis following anaesthesia. Skin tests positive to Vecuronium	Vecuronium, flucloxacillin	Anaesthetic agents, penicillins, cephalosporins
		On another occasion, allergic to penicillin and sulfonamides		Trimethoprim, sulfamethoxazole
Hen	F, 49	Anaphylactoid reaction (bronchospasm, hypotension, skin erythema) on induction of anaesthesia	Anaesthetic agents	Anaesthetic agents
Hem	M, 27	Reaction during anaesthesia in 1994 following marcaine and morphine. Anaphylaxis after being given more morphine	Morphine and other anaesthetic agents	Anaesthetic agents
Sco	F, 47	Anaphylactic reaction during anaesthesia	Vecuronium, fentanyl, propofol, midazolam	Anaesthetic agents
Kea	F,	Severe urticaria after a course of cephalosporins	Cephalexin	Pencillins, cephalosporins, trimethoprim, sulfamethoxazole, minomycin

* Anaesthetic agents: Neuromuscular blocking drugs, thiopentone & various narcotics.

resuspended in distilled water. The drug-EA Sepharose 6B complex was stored at 4°C.

Preparation of Drug-Protein Covalent Conjugates

Human serum albumin (HSA) conjugates of benzylpenicillin (penicillin G), ampicillin, amoxicillin, cephalexin, and cefaclor prepared with water-soluble 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), as well as the control sample of HSA treated with carbodiimide in the absence of drug, have been previously described.(18) Briefly, the drug (30, 30, 10, 10, and 10 mg, respectively) in distilled water (4 mL) was mixed with HSA (20 mg) and EDC (30 mg) and the pH adjusted to 5.5 with 0.1 M HCl. The reaction mixture was incubated at 25°C for 20 h with gentle rocking and dialysed extensively against distilled water for three days.

The conjugates were then adsorbed to nitrocellulose (NC) 6 mm discs (20 mg of complex per disc for 20 h at room temperature) and the remaining free sites on discs were blocked with 0.1% Tween-20 in phosphate-buffered saline (PBS). Control discs, adsorbed with HSA alone (i.e., without drug and EDC), were also prepared and blocked in the same manner as described above. The NC discs were blotted dry and stored at -20°C.

Detection of Drug-Reactive IgE Antibodies by Radioimmunoassay and Inhibition Assay

Antibodies were detected as previously described(9, 17, 18) with some modifications. Briefly, serum (25 L) was incubated for 3 h at room temperature with the drug-solid phase. Sedimented Sepharose conjugates or NC discs were then washed three times with PBS containing 0.05% Tween-20 and 0.05% sodium azide before incubation overnight with about 30,000 cpm of ^{125}I-anti-human IgE. After further washes as above, tubes were counted in a Packard Auto-Gamma Spectrometer. The presence of specific IgE antibodies was determined by the percent radioactive uptake of ^{125}I-anti-human IgE, i.e., percent of counts added.

For inhibition studies, inhibitor solutions were prepared in PBS to keep the pH at about 6.5 - 7.5. Serum (50 μL), appropriately diluted, was incubated for 1 h at room temperature with 50μL of a solution of inhibitor before the addition of the drug-solid phase conjugate. The assay was then continued as described earlier. All inhibition tests were performed in duplicate.

RESULTS

Multiple Drug Recognition by IgE Antibodies in Sera from Allergic Subjects

Results of direct IgE antibody binding studies employing sera from 7 different patients, together with the β-lactam antibiotics amoxicillin, cephalothin, and cephalexin, some other antibacterials, the anaesthetic agents thiopentone and *d*-tubocurarine, the local anaesthetic procaine; promethazine, a phenothiazine antihistamine, and the narcotic morphine, are shown in Table 2. Almost all of the drug-solid phases tested gave positive reactions with each of the sera, except for occasional negative results, for example, promethazine, alcuronium, and vecuronium with serum Kea, thiopentone with Hen and Hem, amoxicillin with serum Hem, and trimethoprim which was positive only with sera Car and Dow. Overall, reactions were particularly strong with morphine. Reactions with the control solid phases, ethanolamine-Sepharose and HSA alone were as expected viz. absent or very weak positives but the EDC-activated-HSA used as a control for the drug-HSA conjugates, prepared with EDC, showed clear, strong binding to IgE antibodies in all 7 sera. Control sera, consisting of sera from cord blood (used as IgE 'free' control), 'normal' healthy non-allergic subjects, subjects allergic to pollens and/or house dust mite but not drugs, and subjects with high total IgE levels, did not show positive reactions with any of the drug- or control-solid phases.

Molecular Basis of IgE Antibody Binding to Drugs. Quantitative Hapten Inhibition Studies

Examination of the structures of the drugs and other chemicals that reacted with the IgE antibodies in the patients' sera and demonstration of antibody reactivity with the carbodiimide (EDC)-activated solid phases, suggested that a common group(s) was being recognized, both on the product of the EDC-activated-HAS, and on at least some of the drugs. Carbodiimide coupling of a drug to protein produces drug-protein complex and drug-acylurea derivatives (Scheme 1).(19) Likewise, activation of protein by EDC in the absence of drug produces protein-protein conjugate, together with corresponding acylureas as side products (Scheme 2).(20) While the drug-acylurea side products are water soluble and dialysable, the larger protein derivatives of acylurea are retained in the reaction product mixture. These acylurea side products contain a dimethylamino group which is, thus, available for reaction with complementary antibodies. Since the

Table 2. Recognition of a Range of Structurally and Pharmacologically Different Drugs by IgE Antibodies in Sera from Subjects Exhibiting Multiple Drug Allergies

Drug/Chemical conjugate	Mean of RU (%) of cord & normal sera controls	Direct binding radioactive uptake (RU) (%)[a] of ^{125}I-anti-human IgE[b] *with sera*						
		Car	Ley	Dow	Hen	Hem	Sco	Kea
Sepharose conjugated to:								
Ethanolamine	*0.5*	*1.9*	*2.8*	*1.6*	*0.6*	*0.5*	*0.8*	*0.5*
Dimethylethanolamine	*0.3*	**48.3** *(4+)*	**25.9** *(4+)*	nd	**42.4** *(4+)*	**12.5** *(4+)*	**6.4** *(3+)*	0.4 *(–)*
Procaine	*0.4*	**48.8** *(4+)*	nd	nd	**49.3** *(4+)*	**34.0** *(4+)*	**6.7** *(3+)*	1.7 *(1+)*
Promethazine	*0.2*	**5.5** *(3+)*	nd	nd	**1.4** *(1+)*	**1.1** *(1+)*	**2.1** *(2+)*	0.3 *(–)*
Morphine	*0.8*	**50.0** *(4+)*	**45.1** *(4+)*	**46.8** *(4+)*	**34.1** *(4+)*	**43.1** *(4+)*	**42.2** *(4+)*	**27.9** *(4+)*
Choline	*0.5*	**35.9** *(4+)*	**28.4** *(4+)*	**22.0** *(4+)*	**36.9** *(4+)*	**32.3** *(4+)*	**13.6** *(4+)*	**2.5** *(1+)*
Alcuronium	*0.6*		**16.4** *(4+)*	**5.8** *(2+)*	**2.1** *(+/–)*	**30.8** *(4+)*	**3.2** *(1+)*	0.7 *(–)*
Vecuronium	*0.5*	**9.0** *(3+)*	3.2 *(–)*	**7.6** *(3+)*	1.1 *(–)*	**4.5** *(2+)*	**2.8** *(1+)*	0.4 *(–)*
d-Tubocurarine	*0.6*	**46.0** *(4+)*	**24.8** *(4+)*	nd	**40.8** *(4+)*	**19.1** *(4+)*	**8.4** *(3+)*	**7.7** *(3+)*
Triethylcholine	*0.3*	**31.5** *(4+)*	**15.5** *(4+)*	nd	**30.5** *(4+)*	**2.9** *(2+)*	**2.6** *(2+)*	**1.4** *(1+)*
Thiopentone	*1.2*	**18.6** *(3+)*	**12.8** *(2+)*	**21.7** *(3+)*	3.3 *(–)*	3.2 *(–)*	**6.0** *(1+)*	**4.4** *(1+)*
Trimethoprim	*0.5*	**9.7** *(3+)*	3.0 *(–)*	**11.7** *(4+)*	0.4 (–)	0.5 *(–)*	0.8 *(–)*	0.3 *(–)*
Tetracycline	*0.5*	**15.3** *(4+)*	**11.6** *(3+)*	nd	1.5 *(–)*	**2.7** *(1+)*	**8.3** *(3+)*	**2.6** *(1+)*
Pipemidic acid	*1.0*	**37.3** *(4+)*	**48.0** *(4+)*	nd	**46.0** *(4+)*	**4.4** *(1+)*	**38.4** *(4+)*	**6.5** *(2+)*
Ofloxacin	*0.5*	**45.9** *(4+)*	**43.8** *(4+)*	nd	**25.4** *(4+)*	**25.8** *(4+)*	**51.4** *(4+)*	1.9 *(+/–)*
Cephalothin	*1.2*	**15.0** *(3+)*	**22.1** *(3+)*	nd	**4.5** *(1+)*	2.4 *(–)*	**4.6** *(1+)*	1.9 *(–)*
Cephalexin	*0.8*	**25.2** *(4+)*	**17.4** *(3+)*	nd	**5.8** *(2+)*	**3.7** *(1+)*	**7.0** *(2+)*	**3.8** *(1+)*
Cefaclor	*0.5*	**21.8** *(4+)*	**20.9** *(4+)*	nd	**2.6** *(1+)*	**3.0** *(2+)*	**7.6** *(3+)*	**4.1** *(2+)*

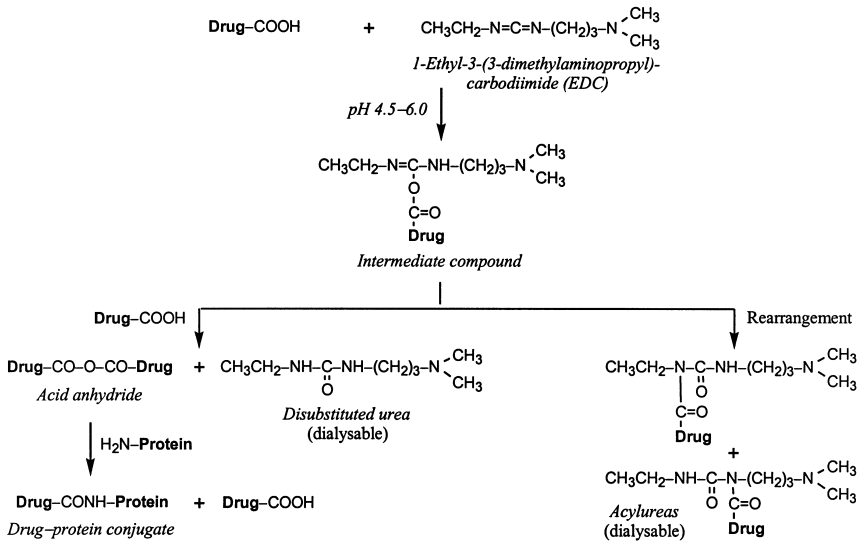
HSA conjugated to:								
HSA alone	*0.3*	*2.7*	*7.8*	*4.4*	*0.7*	*1.1*	*1.0*	*0.7*
EDC-activated-HSA[c]	*0.4*	**32.7** *(4+)*	**37.6** *(4+)*	**38.5** *(4+)*	**50.9** *(4+)*	**24.7** *(4+)*	**43.8** *(4+)*	**34.0** *(4+)*
Penicillin G	*0.5*	**11.6** *(3+)*	**22.7** *(4+)*	**30.4** *(+)*	**55.4** *(4+)*	**17.4** *(4+)*	**22.8** *(4+)*	**20.2** *(4+)*
Ampicillin	*0.6*	**8.6** *(2+)*	**9.9** *(1+)*	4.7 *(–)*	**4.0** *(2+)*	2.1 *(–)*	**2.5 (+/–)**	**35.5** *(4+)*
Amoxicillin	*0.5*	**42.9** *(4+)*	**14.3** *(3+)*	**38.7** *(4+)*	**22.0** *(4+)*	1.5 *(–)*	**3.9** *(2+)*	**16.0** *(4+)*
Cephalexin	*0.5*	**37.5** *(4+)*	**41.6** *(4+)*	**38.4** *(4+)*	**46.9** *(4+)*	**18.9** *(4+)*	**36.8** *(4+)*	**27.1** *(4+)*
Cefaclor	*0.5*	**8.9** *(3+)*	**10.7** *(2+)*	**30.5** *(4+)*	2.0 (+/–)	1.1 *(–)*	2.2 (+/–)	**32.9** *(4+)*

[a] After deducting the background (ethanolamine for Sepharose conjugates and HSA alone for HSA conjugates), a radioactive uptake greater than three times the uptake obtained with cord or normal serum was considered to indicate a positive reaction with the drug and is highlighted in the table. The score criteria are as follows:

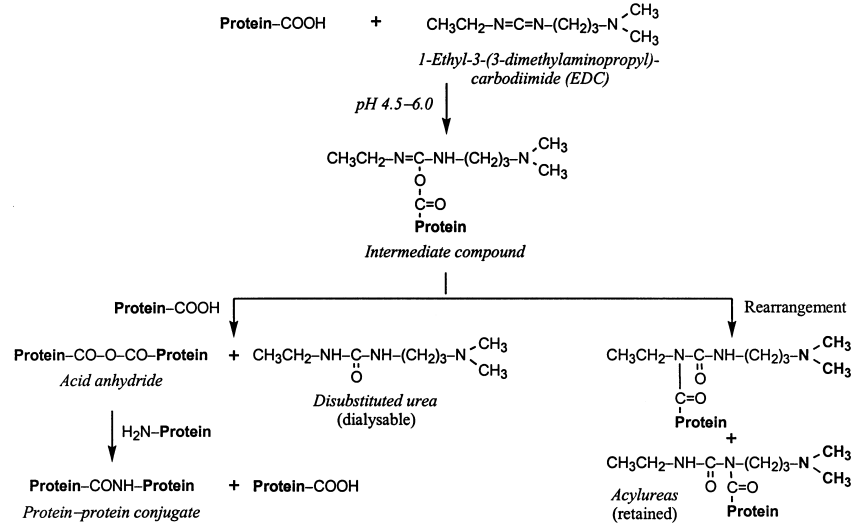
Range	*Score*	*Range*	*Score*
< 2.5 x cord	–	5–10 x cord	2+
2.5–3 x cord	+/–	10–20 x cord	3+
3–5 x cord	1+	> 20 x cord	4+

[b] Directly proporational to the uptake of IgE antibodies.

[c] Acylureas together with protein-protein conjugate, see Scheme 2.



Scheme 1. Reactions involved in the carbodiimide coupling of a drug to protein.



Scheme 2. Reactions involved in the activation of protein by carbodiimide in the absence of drug.

participation of such groups as allergenic determinant structures involved in IgE antibody binding is well established,(9, 13, 21, 22) preliminary investigations were directed at the possibility that tertiary amino groups were a common recognition feature on many, or some, of the IgE reactive drugs.

Employing EDC-activated-HSA on NC discs as the solid phase, preliminary inhibition studies were undertaken, using sera from the multi-drug reactive patients together with selected amines and drugs containing substituted amino groups. Figures 1, 2, and 3 show the results obtained in the inhibition studies with four of the sera, Car, Hen, Ley, and Kea. With substituted simple amines, trimethylamine was the most potent inhibitor. Dimethylamine was clearly inhibitory in the range 100-1000 nmol/tube with 3 of the sera but poorly inhibitory with serum Kea, while methylamine was without activity (Fig. 1 a–d). With ethanolamines as inhibitors, the order of activity was dimethylethanolamine > methylethanolamine > ethanolamine (Fig. 1 e–h).

The phenothiazine promethazine and local anaesthetic procaine (Fig. 2 a–d) and the narcotics morphine and nalorphine (Fig. 2 e–h) demonstrated at least some degree of complementarity to the IgE combining sites, although, again, inhibition was less marked with serum Kea (Fig. 2 d, h).

The NMBD atracurium proved to be a very potent inhibitor with all four sera and, while another NMBD, alcuronium, and the quaternary ammonium compound choline chloride, were good inhibitors of IgE in serum Car, these compounds were only weakly active with sera Hen, Ley, and Kea (Fig. 3 a–d). The quinolone antibacterials, ofloxacin and ciprofloxacin, both showed clear-cut activity with sera Car, Hen, and Ley, while only the former drug showed strong inhibition (24 nmol for 50 % inhibition) with serum Kea. The broad spectrum antibacterial tetracycline showed much weaker, but clear inhibition with sera Hen, Ley, and Kea in the range 100-1000 nmol/tube (Fig. 3 e–h).

On the basis of these preliminary results on the likely substituted amino group specificity of the IgE antibodies, more comprehensive quantitative hapten inhibition investigations were undertaken. Detailed findings with one of the sera, serum Car, are presented in Figures 4-9. Of the simple amines tested (Fig. 4), tertiary substituted amines with methyl or ethyl substituents were the most active. Substitution of trimethylamine with one or two ethyl groups did not reduce the 50 per cent inhibitory potency (8 nmol/tube) but triethylamine was significantly less active and activity decreased markedly with increased alkyl chain length reflected in the results obtained with tripropyl and tributyl amines.

The secondary dimethyl and diethyl amines were weakly inhibitory and, again, increased alkyl chain length produced a marked fall in activity. Primary amines were without activity. With the range of ethanolamines

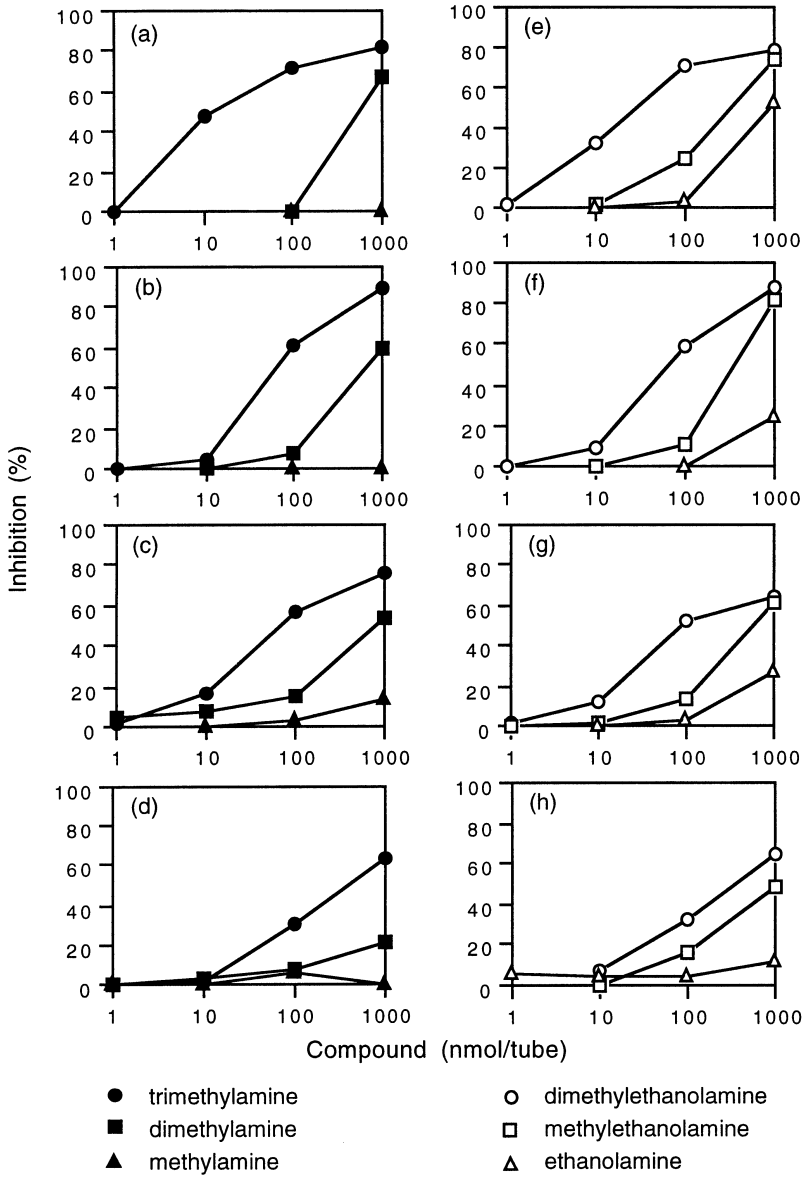


Figure 1. Inhibition of IgE antibody binding to EDC-activated-HSA solid phase in sera (a, e) Car; (b, f) Hen; (c, g) Ley, and (d, h) Kea by [a, b, c, d] amines trimethylamine, dimethylamine and methylamine; and by [e, f, g, h] ethanolamines dimethylethanolamine, methylethanolamine, and ethanolamine. Serum Car, Hen, Ley, and Kea were used at dilutions of 1:100, 1:50, 1:50, and 1:10, respectively.

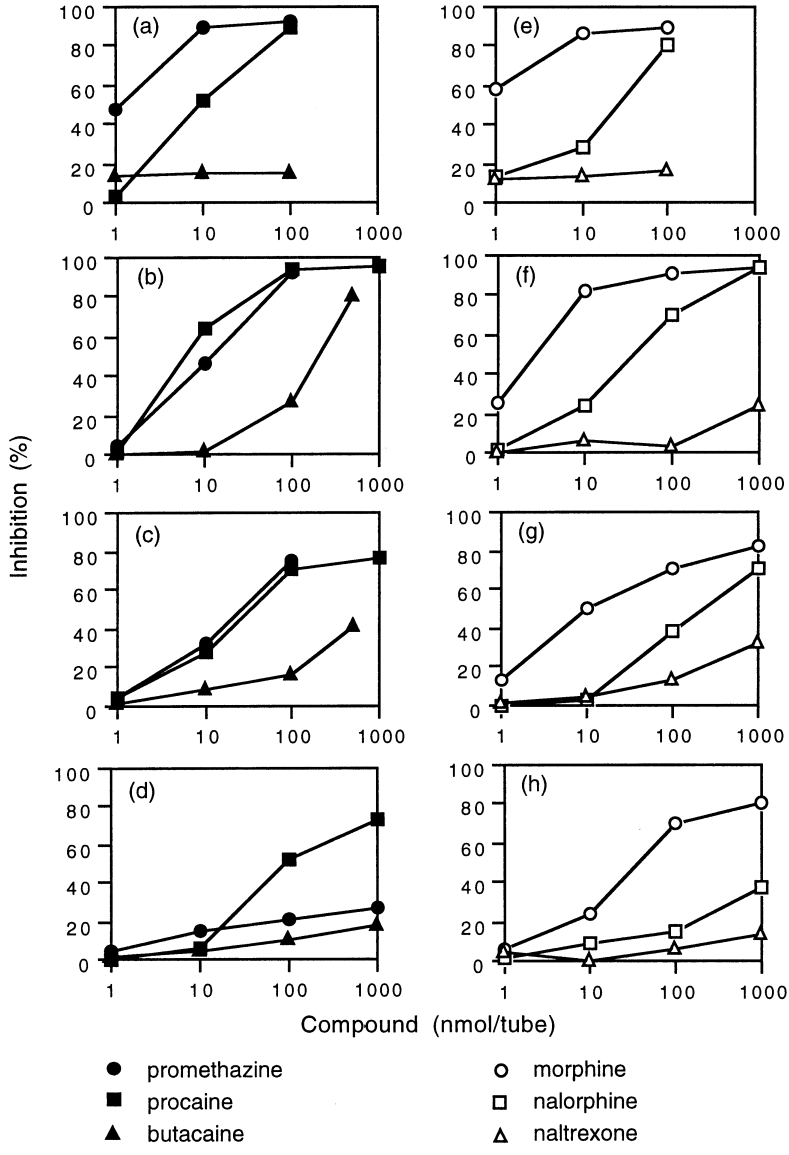


Figure 2. Inhibition of IgE antibody binding to EDC-activated-HSA solid phase in sera (a, e) Car; (b, f) Hen; (c, g) Ley, and (d, h) Kea by [a, b, c, d] antihistamine promethazine, and local anaesthetics procaine and butacaine; and by [e, f, g, h] narcotic analgesic morphine, and antagonists nalorphine and naltrexone. Serum Car, Hen, Ley, and Kea were used at dilutions of 1:100, 1:50, 1:50, and 1:10, respectively.

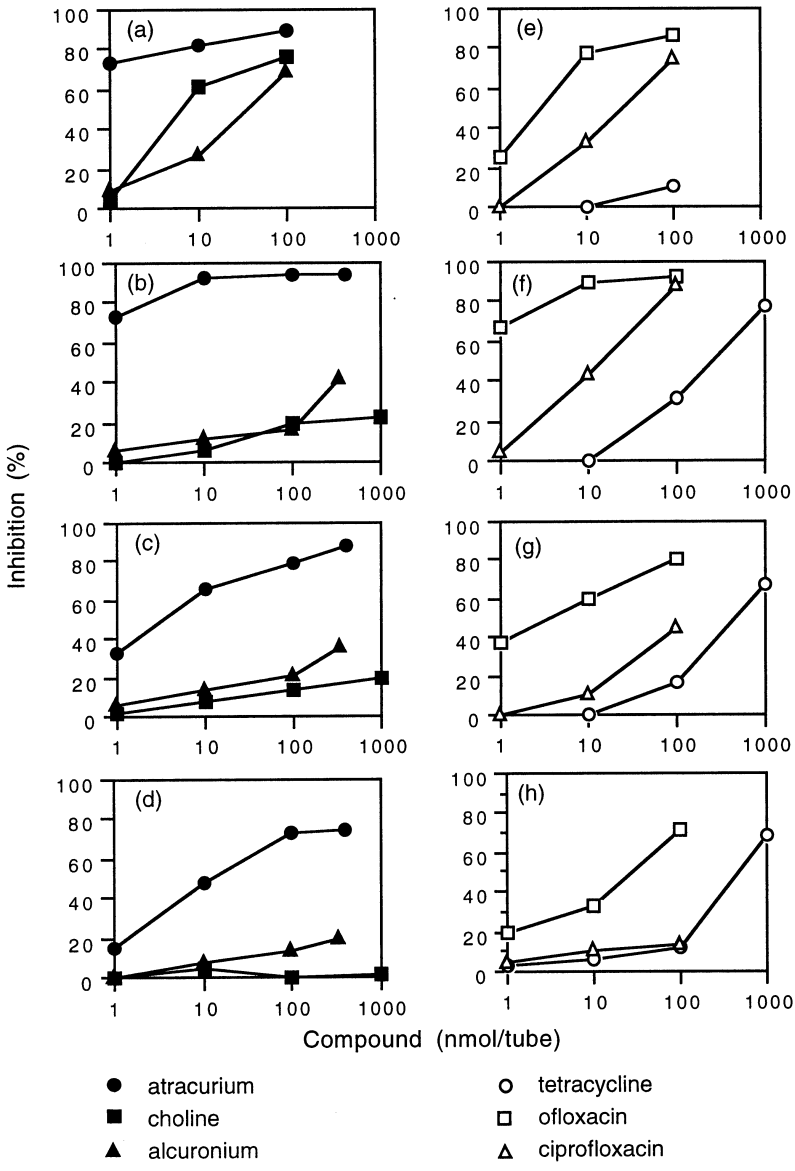


Figure 3. Inhibition of IgE antibody binding to EDC-activated-HSA solid phase in sera (a, e) Car; (b, f) Hen; (c, g) Ley, and (d, h) Kea by [a, b, c, d] neuromuscular blocking drugs atracurium and alcuronium, and structural analog choline; and by [e, f, g, h] tetracycline, and quinolones ofloxacin and ciprofloxacin. Serum Car, Hen, Ley, and Kea were used at dilutions of 1:100, 1:50, 1:50, and 1:10, respectively.

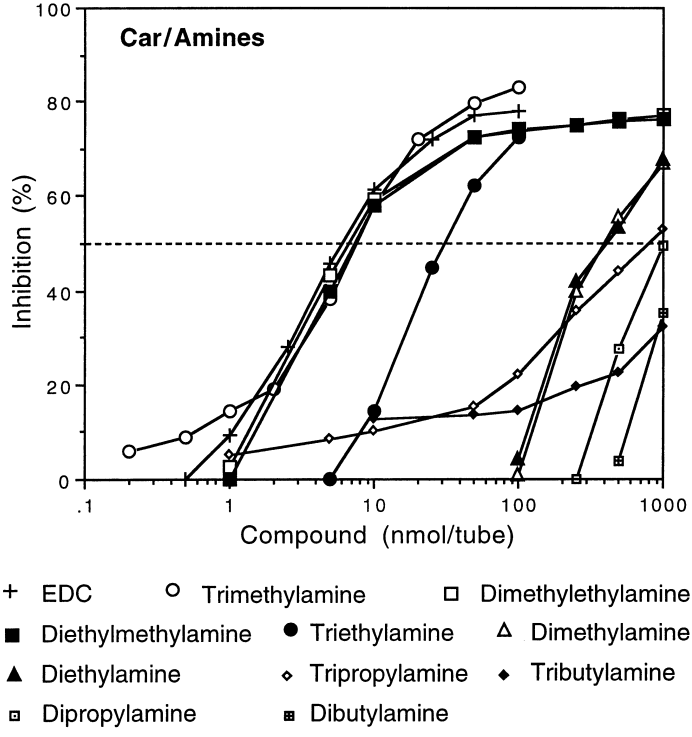


Figure 4. Inhibition of IgE antibody binding to EDC-activated-HSA solid phase in serum Car by EDC and simple amines trimethylamine, dimethylethylamine, diethylmethylamine, triethylamine, dimethylamine, diethylamine, tripropylamine, tributylamine, dipropylamine, and dibutylamine. Serum Car was used at a dilution of 1:100.

tested, a similar pattern was observed. Presence of a tertiary methylamino group was related to the highest inhibitory potency, and this decreased progressively with increasing alkyl chain length and with the employment of secondary and primary amino compounds (Fig. 5).

The importance of the dimethylamino and, to a lesser extent, the diethylamino group in IgE recognition was further demonstrated with the pharmacologically active neostigmine and promethazine, and local anaesthetics tetracaine and procaine (Fig. 6), and with the narcotics and narcotic antagonists (Fig. 7). Interestingly, lignocaine which, like procaine, contains a diethylamino group, but an amide instead of a primary amino, was without activity (Fig. 6). Marked decreases in inhibitory activity were observed in the narcotic group by replacement of the N-methyl

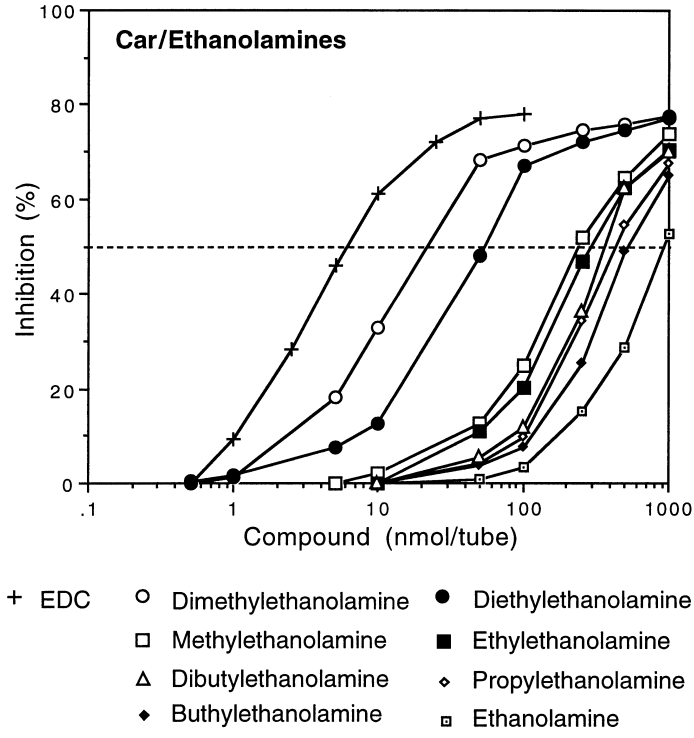


Figure 5. Inhibition of IgE antibody binding to EDC-activated-HSA solid phase in serum Car by EDC and ethanolamines dimethylethanolamine, diethylethanolamine, methylethanolamine, ethylethanolamine, dibutylethanolamine, propylethanolamine, butylethanolamine, and ethanolamine. Serum Car was used at a dilution of 1:100.

substituent (morphine, codeine) with an N-allyl (nalorphine, naloxone), an N-cyclopropylmethyl (naltrexone), or an N-phenylethyl group (fentanyl) (Fig. 7).

NMBDs containing one or more methylammonium, dimethylammonium, or trimethylammonium groups were very strong inhibitors, while activity decreased when the quaternary ammonium group was changed to allylammonium (rocuronium, alcuronium) and triethylammonium (triethylcholine, gallamine) (Fig. 8).

The pattern of preferential recognition of tertiary methylamino groups was shown again with the quinolone antibacterials examined, where substitution of methyl groups reduced inhibitory activity (Fig. 9).

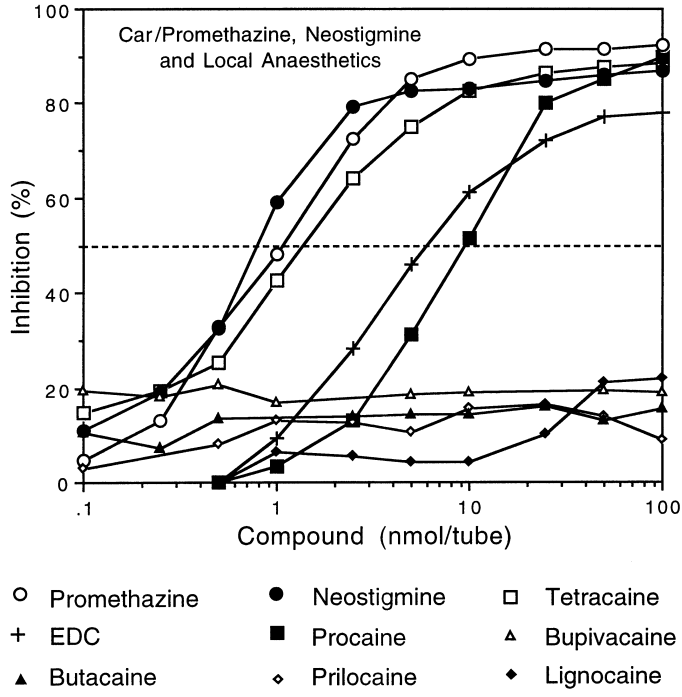


Figure 6. Inhibition of IgE antibody binding to EDC-activated-HSA solid phase in serum Car by cholinergic agent neostigmine, antihistamine promethazine, EDC, and local anaesthetics tetracaine, procaine, bupivacaine, butacaine, prilocaine, and lignocaine. Serum Car was used at a dilution of 1:100.

Summary of Structure-Activity Relationships Derived from Antibody Combining Site Specificity Studies. Quantitative Comparison of Inhibition Potencies of Drugs and Other Chemicals

Inhibition potencies derived from detailed quantitative hapten inhibition experiments with serum Car (Figs 4–9) are set out in Table 3. Results with selected key compounds used with sera Hen, Ley and Kea are also included. These results, considered in conjunction with the findings shown in Figs 4-9, emphasize the importance of tertiary methylamino and quaternary methylammonium groups as complementary structures to the IgE antibodies in the sera of the multi drug-reactive subjects.

Although procaine was a good inhibitor of IgE antibody binding (Figs 2, 6; Table 3), other local anaesthetics, containing an amide group, viz.,

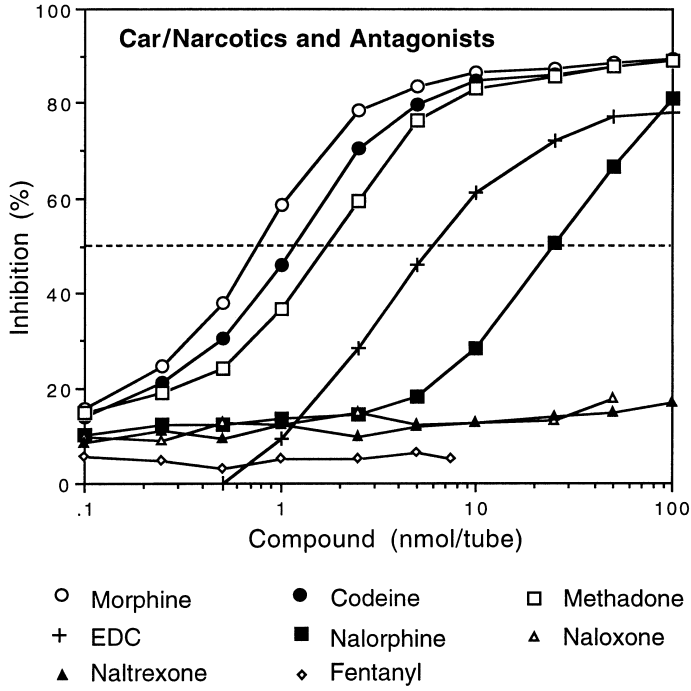


Figure 7. Inhibition of IgE antibody binding to EDC-activated–HSA solid phase in serum Car by narcotic analgesics morphine, codeine, methadone, and fentanyl; and antagonists to narcotics nalorphine, naloxone, and naltrexone; and EDC. Serum Car was used at a dilution of 1:100.

lignocaine, prilocaine, butacaine, and bupivacaine were very weakly active or inactive. This difference in inhibitory potencies was highlighted by results with procaine and lignocaine, both of which contain a diethylamino group. IgE antibody binding was also weak or absent with tetracycline, doxycycline, and minocycline, each of which contains an N,N-dimethylamino together with an amide group (Fig. 3; Table 3). Near neighbour effects promoting weaker antigenic recognition may also account for apparent anomalous results seen with some narcotics (Figs 2, 7; Table 3) and quinolones (Figs 3, 9; Table 3).

Although nalorphine and naloxone each contain a tertiary allylamino group, naloxone also has a hydroxyl adjacent to the substituted amino group. Likewise, although pipemidic acid and cinoxacin each have a tertiary ethylamino group, it seems likely that the cinnoline nucleus present in the latter quinolone contributes to its poorer inhibitory potency.

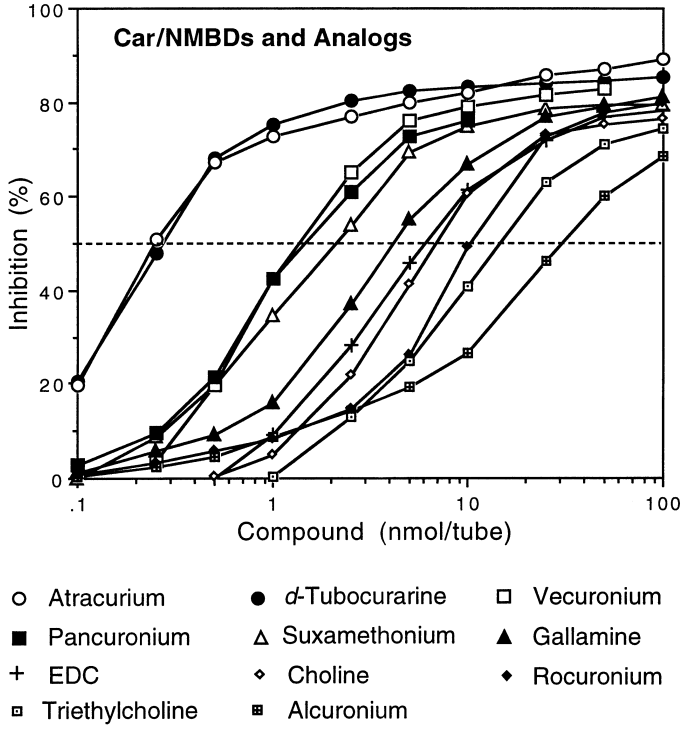


Figure 8. Inhibition of IgE antibody binding to EDC-activated-HSA solid phase in serum Car by neuromuscular blocking drugs atracurium, *d*-tubocurarine, vecuronium, pancuronium, suxamethonium, gallamine, rocuronium, and alcuronium; structural analogs choline, and triethylcholine; and EDC. Serum Car was used at a dilution of 1:100.

β-Lactam drugs, benzylpenicillin, ampicillin, amoxicillin, cephalexin, and cefaclor did not inhibit binding of any of the sera to the EDC activated-HSA solid phase. By contrast, these compounds, to greater or lesser extent, inhibited binding of serum IgE antibodies to β-lactam solid phases such as cephalexin-Sepharose, indicating a separate population(s) of antibodies to the substituted amino group-reactive IgE antibodies.

DISCUSSION

Direct binding and inhibition studies on the specificities of the IgE antibodies found in the sera of subjects exhibiting allergic sensitivities to a

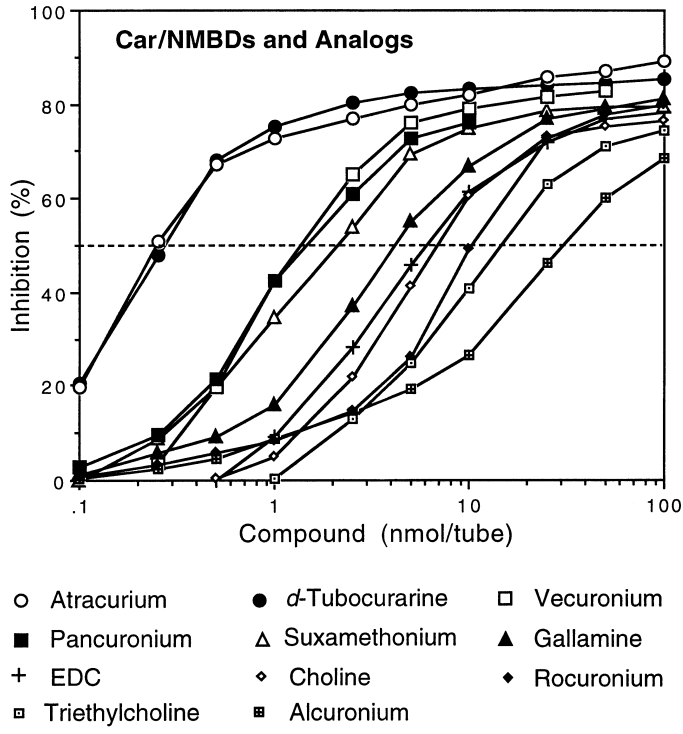


Figure 9. Inhibition of IgE antibody binding to EDC-activated-HSA solid phase in serum Car by quinolones ofloxacin, pefloxacin, pipemidic acid, ciprofloxacin, flumequine, and cinoxacin; and EDC. Serum Car was used at a dilution of 1:100.

variety of pharmacologically different drugs were undertaken in an effort to elucidate the molecular basis of the multiple drug recognition. When structures of the drugs and other chemicals employed were closely scrutinized and correlated with their antibody inhibitory activities, a clear correlation between some small, common structural features on the compounds and their inhibitory potencies emerged. Recognition of substituted amino and ammonium groups appears to explain the wide ranging drug recognition of IgE antibodies in the sera of the patients with multiple drug reactivities. Tertiary mono-, di- and trialkylamino groups, and quaternary mono-, di-, and trialkylammonium groups were all recognized, but only if the alkyl groups were 'small', viz., methyl or, perhaps, ethyl. In other words, inhibitory potencies of the compounds were found to be inversely proportional to

the size of the N-alkyl substituents. The observed relative degree of antigenic complementarity to the antibody combining sites can be summarized as:

a. With tertiary amino groups:
$>N(CH_3)$; $-N(CH_3)_2$; $N(CH_3)_3$ > $>N(C_2H_5)$; $-N(C_2H_5)_2$; $N(C_2H_5)_3$ > $>N(CH_2CH=CH_2)$

b. With quaternary ammonium groups:
$>N^+(CH_3)-$; $>N^+(CH_3)_2$ > $-N^+(CH_3)_3$ > $>N^+(C_2H_5)_2$ > $>N^+(CH_2CH=CH_2)-$

Primary amino ($-NH_2$), secondary amino (–NHR) and tertiary amino ($-NR_2$ with R = 'large' alkyl) groups showed no inhibitory activity. The presence of an amide group appears to diminish antibody recognition and interaction (c.f. inhibition results of procaine and lignocaine) and such near neighbour effects may also account for unexpected reductions in antibody recognition seen with structurally similar narcotic and quinolone drugs.

This recognition specificity does not, however, explain antibody and clinical sensitivities to a number of other drugs, in particular, the β-lactam antibiotics. Failure of compounds such as benzylpenicillin, ampicillin, cephalexin, etc., to inhibit binding of IgE antibodies to the EDC-activated-HSA solid phase, but inhibition of antibody binding to β-lactams suggests that separate populations of antibodies may coexist in the sera of the subjects studied here. This finding accords with the results of Khoury and Warrington*(8)* who concluded in their study of the multiple drug allergy syndrome that the development of penicillin allergy and non-β-lactam allergy are not associated and occur independently.

Our findings are consistent with the observations of Harris and Harris,*(5)* who studied a patient who had allergic reactions to a number of antibiotics during treatment of recurrent oral infection. The offending drugs, erythromycin, tetracycline, lincomycin, diphenhydramine, and colestyramine, but not cephalexin, all have methylamino or quaternary ammonium groups.

In summary, findings reported here showed that IgE antibody recognition of tertiary alkyl (in particular, methyl) amino groups occurred in subjects with allergic sensitivities to a range of pharmacologically different drugs. These results indicate that the antibody recognition spectra in sera from at least some subjects with multiple drug allergies may include antibodies with wide ranging cross-reactivity with many drugs by virtue of recognition of tertiary amino and/or quaternary ammonium groups which are present in many different pharmacologically active compounds. In addition to such cross-reactive IgE antibodies, separate populations of antibodies to other non cross-reacting drugs may be present.

Table 3. Correlation Between Small, Common Structural Features on Drug Molecules and the Inhibition of IgE Antibody Binding to EDC-activated-HSA Solid Phase Seen with EDC, Amines, Ethanolamines, and a Variety of Pharmacologically and Structurally Different Drugs

Compound	Alkylamino or Alkylammonium group(s)[a]	Structure[b]	Amount (nmol/tube) of compound needed for 50% inhibition in serum			
			Car	Hen	Ley	Kea
EDC	*tert dimethylamino*	$-N(CH_3)_2$	6			
SIMPLE AMINES:						
**** Tertiary Amines:***						
Trimethyamine	*tert trimethylamine*	$CH_3-N(CH_3)_2$	9	60	60	380
Dimethylethylamine	*tert dimethylethylamine*	$C_2H_5-N(CH_3)_2$	8			
Diethylmethylamine	*tert diethylmethylamine*	$CH_3-N(C_2H_5)_2$	8			
Triethylamine	*tert triethylamine*	$C_2H_5-N(C_2H_5)_2$	30			
Tripropylamine	*tert tripropylamine*	$C_3H_7-N(C_3H_7)_2$	800			
Tributylamine	*tert tribuylamine*	$C_4H_9-N(C_4H_9)_2$	> 1000	> 1000	> 1000	n.i.[a]
**** Secondary Amines:***						
Dimethylamine	*sec dimethylamine*	$CH_3-NH(CH_3)$	400	620	710	> 1000
Diethylamine	*sec diethylamine*	$C_2H_5-NH(C_2H_5)$	400			
Dipropylamine	*sec dipropylamine*	$C_3H_7-NH(C_3H_7)$	1000			
Dibutylamine	*sec dibutylamine*	$C_4H_9-NH(C_4H_9)$	> 1000	480	1000	n.i.
**** Primary Amines:***						
Methyamine	*prim methylamine*	CH_3-NH_2	n.i.	n.i.	> 1000	n.i.
Ethylamine	*prim ethylamine*	$C_2H_5-NH_2$	n.i.			

ETHANOLAMINES:						
**** Tertiary Amino groups:***						
Dimethyethanolamine	*tert dimethylamino*	$HOCH_2CH_2–N(CH_3)_2$	20	62	90	380
Diethylethanolamine	*tert diethylamino*	$HOCH_2CH_2–N(C_2H_5)_2$	50			
Dibutylethanolamine	*tert dibutylamino*	$HOCH_2CH_2–N(C_4H_9)_2$	350			
**** Secondary Amino groups:***						
Methylethanolamine	*sec methylamino*	$HOCH_2CH_2–NH)CH_3)$	230	350	550	> 1000
Ethylethanolamine	*sec ethylamino*	$COCH_2CH_2–NH(C_2H_5)$	280			
Propylethanolamine	*sec propylamino*	$HOCH_2CH_2–NH(C_3H_7)$	400			
Butylethanolamine	*sec butylamino*	$HOCH_2CH_2–NH(C_4H_9)$	500			
**** Primary Amino group:***						
Ethanolamine	*prim amino*	$HOCH_2CH_2–NH_2$	900	> 1000	> 1000	> 1000
NEOSTIGMINE, PROMETHAZINE and LOCAL ANAESTHETICS:						
Neostigmine	*tert diemthylamino + quat trimethylammonium*	$–N(CH_3)_2 + –N^+(CH_3)_3$	0.8			
Promethazine	*tert dimethylamino + tert amino*	$–N(CH_3)_2 + > N–$	1	5.5	14	> 1000
Tetracaine	*tert dimethylamino + sec butylamino*	$–N(CH_3)_2 + – NH(C_4H_9)$	1.6			
Procaine	*tert diethylamino + prim amino*	$–N(C_2H_5)_2 + –NH_2$	10	11	20	80
Lignocaine	*tert diethylamino + sec amide*	$–N(C_2H_5)_2 + –CONH–$	n.i.			
Prilocaine	*sec propylamino + sec amide*	$–NH(C_3H_7) + –CONH–$	n.i.			
Butacaine	*tert dibutylamino + prim amino*	$–N(C_4H_9)_2 + –NH_2$	n.i.	200	1000	> 1000
Bupivacaine	*tert butylamino + sec amide*	$> N(C_4H_9) + –CONH–$	n.i.			

(*continued*)

Table 3. *Continued*

Compound	Alkylamino or Alkylammonium group(s)[a]	Structure[b]	Amount (nmol/tube) of compound needed for 50% inhibition in serum			
			Car	Hen	Ley	Kea
NARCOTICS and ANTAGONISTS:						
Morphine	*tert methylamino*	$>N(CH_3)$	0.8	0.8	10	36
Codeine	*tert methylamino*	$>N(CH_3)$	1.1			
Methadone	*tert dimethylamino*	$-N(CG_3)_2$	1.6			
Nalorphine	*tert allylamino*	$>N(CH_2CH=CH_2)$	25	35	220	>1000
Naloxone	*tert allylamino + 14-hydroxy*	$>N(CH_2CH=CH_2)+14–OH$	n.i.			
Naltrexone	*tert cyclopropylmethylamino*	$>N(CH_2–\triangleleft)$	n.i.	>1000	>1000	>1000
Fantanyl	*tert phenylethylamino + tert amide*	$>N(CH_2CH_2–C_6H_5)+–CON<$	n.i.			
NEUROMUSCULAR BLOCKING DRUGS and ANALOGS:						
Atracurium	*2 quat methylammonium*	$(2\times)>N^+(CH_3)$	0.25	<1	3	10.5
d-Tubocurarine	*1 quat dimethylammonium + 1 tert methylammonium*	$>N^+(CH_3)_2+>N^+H(CH_3)$	0.25			
Vecuronium	*1 quat methylammonium + 1 tert pipridinium*	$>N^+(CH_3)–+>NH\ \ H–$	1.4			
Pancuronium	*2 quat methylammonium*	$(2\times)>N^+(CH_3)–$	1.5			
Suxamethonium	*2 quat trimethylammonium*	$(2\times)–N^+(CH_3)_3$	2			
Gallamine	*3 quat triethylammonium*	$(3\times)–N^+(C_2H_5)_3$	4			
Choline	*1 quat trimethylammonium*	$–N^{++}(CH_3)_3$	10	>1000	>1000	n.i.
Rocuronium	*1 quat allylammonium + 1 tert amino*	$>N^+(CH_2CH=CH_2)–+\ \ >N–$	10			
Triethylcoline	*1 quat triethylammonium*	$–N^+(C_2H_5)_3$	15			
Alcuronium	*2 quat allylammonium*	$(2\times)>N^+(CH_2CH=CH_2)–$	30	520	1000	>1000

TETRACYCLINES:						
Tetracycline	*1 tert dimethylamino + prim amide*	$-N(CH_3)_2 + -CONH_2$	n.i.	220	450	450
Doxycycline	*1 tert dimethylamino + prim amide*	$-N(CH_3)_2 + -CONH_2$	n.i.			
Minocycline	*2 tert dimethylamino + prim amide*	$(2x)-N(CH_3)_2 + -CONH_2$	ni.i			
QUINOLONES:						
Ofloxacin	*tert methylamino + tert amino*	$> N(CH_3) + \ > \ N -$	2.5	< 1	3.1	24
Pefloxacin	*tert methylamino + tert ethylamino*	$> N(CH_3) + > N(_2H_5)$	6			
Pipemidic acid	*tert ethylamino + sec amino*	$> N(C_2H_5) + > NH$	18			
Ciprofloxacin	*tert cyclopropylamino + sec amino*	$> N-\triangleleft + > NH$	18	14	140	> 1000
Flumequine	*tert amino*	$> N-$	25			
Cinoxacin	*tert ethylamino + cinnoline nucleus*	$> N(C_2H_5)$ + cinnoline nucleus	n.i.			

[a] Abbreviations: prim, primary; sec, secondary; tert, tertiary; quat, quaternary; n.i., no inhibition observed.
[b] Except for amines and ethanolamines, only interested groups of the molecules are shown.

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