

# A new radioimmunoassay using a commercially available solid support for the detection of IgE antibodies against muscle relaxants

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*It is well established that muscle-relaxant drugs may be responsible for anaphylactoid reactions during anesthesia. In this study, we developed an in vitro test with a commercially available solid phase for the detection of specific IgE directed to the tertiary or quaternary ammonium groups of neuromuscular-blocking drugs. The solid-phase complex was p-aminophenylphosphoryl-choline (PAPPC) immobilized on agarose, and an RIA was performed with an antihuman IgE labeled with <sup>125</sup>I. The results, expressed as the percentage of <sup>125</sup>I-labeled anti-IgE linked to the solid phase, were at  $0.41 \pm 0.19$  for 34 control sera from nonallergic healthy adults, with an upper limit estimated at 1%. The values obtained with the sera of 31 allergic patients ranged from 0.6% to 41% with a sensitivity of 97%. The specificity and the positive predictive value of the PAPPC RIA were 97% and 94%, respectively. These results were compared with results of other RIAs with morphine, trimethylamine, triethylamine immobilized on epoxy-activated Sepharose, and choline hydrochloride immobilized on Sepharose (quaternary ammonium Sepharose RIA) and with Phadebas RAST succinylcholine and Phadebas RAST alcuronium. The PAPPC RIA appears to be the most efficient test to screen sera for the presence of IgE antibodies directed to neuromuscular-blocking drugs. One major advantage is that this solid phase is commercially available and ready to use. This advantage will improve the accuracy in the comparison of the results with results from different laboratories. (J ALLERGY CLIN IMMUNOL 1992;90:153-9.)*

**Key words:** Radioimmunoassay, IgE, muscle relaxants, allergy

Anaphylaxis to intravenous agents used during GA occurs in approximately one in 5000 to 15,000 operations,<sup>1</sup> and NMBDs were responsible for 51% of these adverse reactions.<sup>2</sup>

There is considerable evidence for a role of IgE antibodies directed to these drugs, and it has been clearly demonstrated that the NMBDs bind specifically to complementary IgE antibodies in the allergic patients' sera via their quaternary or tertiary am-

### Abbreviations used

|        |                                 |
|--------|---------------------------------|
| GA:    | General anesthesia              |
| NMBD:  | Neuromuscular blocking drug     |
| PAPPC: | p-Aminophenylphosphoryl-choline |
| PBS:   | Phosphate-buffered saline       |
| QAS:   | Quaternary ammonium Sepharose   |
| TEA:   | Triethylamine                   |
| TMA:   | Trimethylamine                  |

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Received for publication Oct. 30, 1991.

Revised March 10, 1992.

Accepted for publication March 12, 1992.

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nium groups.<sup>3-6</sup> These common epitopes explain the cross-reactivity among the different NMBDs.

We have developed an in vitro test with a commercially available solid phase for the detection of specific IgE directed to the tertiary and/or quaternary ammonium groups of NMBDs in sera from subjects sensitized to these drugs, whatever the NMBD involved. In this study, we present the results obtained with a new RIA, performed with PAPPC immobilized

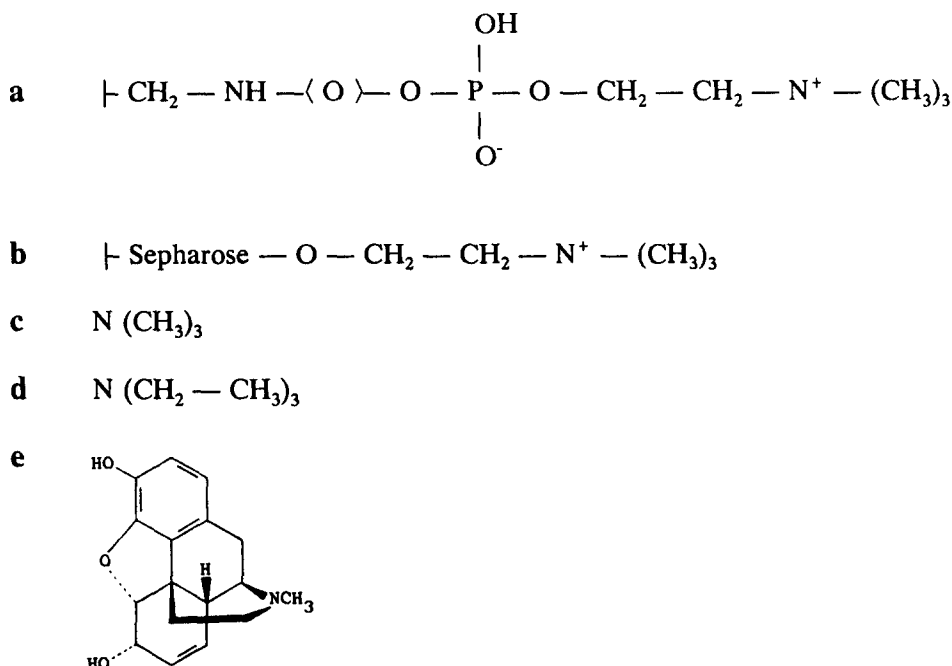


FIG. 1. Chemical structure of *a*, PAPPC immobilized to 6% beaded agarose; *b*, choline hydrochloride linked to Sepharose (QAS Sepharose); *c*, TMA; *d*, TEA; *e*, morphine.

on agarose. Comparisons with results obtained with other RIAs with morphine, TMA, and TEA immobilized on epoxy-activated Sepharose,<sup>7</sup> choline hydrochloride immobilized on Sepharose, noted quaternary ammonium Sepharose RIA or QAS RIA,<sup>8</sup> and with Phadebas RAST alcuronium and Phadebas RAST succinylcholine are presented. Our test reveals the highest sensitivity (97%) for the detection of specific IgE antibodies directed against NMBDs in sera of patients who have developed an anaphylactoid reaction during GA.

## MATERIAL AND METHODS

### Patients and sera

The 31 patients (three male and 28 female patients) included in this study had experienced anaphylactoid reactions during GA and were referred to the Department of Intensive Care (Dr. Motin, E. Herriot Hospital, Lyon, France) for evaluation of anaphylaxis. The muscle relaxants incriminated were succinylcholine (seven patients), gallamine (four patients), alcuronium (eight patients), pancuronium (four patients), vecuronium (seven patients), and atracurium (one patient). The diagnosis was established from 1 to 10 months after the administration of an NMBD for 18 patients, and from 2 to 15 years after GA in 13 other cases. All subjects had positive intradermal tests to the NMBD administered during GA.

The control group was comprised of allergic subjects (13 male and 24 female subjects) who experienced reactions during GA to other drugs than to muscle relaxants and had negative intradermal tests to muscle relaxants, and 34 non-

allergic, healthy adults (four male and 30 female adults) from the laboratory staff members.

Serum samples were stored at  $-20^{\circ}\text{C}$  until use. (Ethylenediaminetetraacetic acid was avoided for the collection of the blood specimens.)

### Drugs and chemicals

TMA hydrochloride, TEA hydrochloride, succinylcholine chloride, and PBS were from Sigma Chemical Co. (St. Louis, Mo.), morphine hydrochloride from Rhône-Poulenc (Livron, France), and atracurium besylate (Tracrium) from Wellcom SA (Paris, France). Vecuronium and pancuronium bromide were provided by Organon-Teknika (Fresnes, France) and alcuronium dichloride by Hoffmann-LaRoche (Basel, Switzerland).

### Preparation of solid-phase complexes

PAPPC immobilized on cross-linked 6% beaded agarose (Fig. 1), ready to use, was commercially available from Pierce Chemical Co. (Rockford, Ill.).

Morphine, TMA, and TEA hydrochloride (Fig. 1) were covalently coupled to epoxy-activated Sepharose 6B (Kabi-Pharmacia, Les Llis France) via the lone pair of electrons on the nitrogen atom to form a stable alkylamine linkage as previously described by Harle et al.<sup>7</sup> Phadebas RAST alcuronium and Phadebas RAST succinylcholine were obtained from Kabi-Pharmacia. Choline bicarbonate was used for preparing the Phadebas RAST succinylcholine, and the covalent coupling to the solid phase (paper) was achieved by use of divinyl sulfone. QAS Sepharose (quaternary ammonium Sepharose) was prepared by an ether linkage of choline hydrochloride to galactose units of Sepharose in the

absence of any spacer<sup>6</sup>; the final formula of the reactive group is illustrated in Fig. 1.

### Detection of IgE antibodies directed to NMBDs by RIA

The detection of IgE antibodies against NMBDs was performed with the solid-phase complexes (PAPPC-agarose and morphine-, TMA-, and TEA-Sepharose), according to the protocol described by Baldo and Fisher<sup>3</sup> with antihuman IgE labeled with <sup>125</sup>I. Serum (50 µl) was added to the solid phase (6 mg) and incubated at room temperature for 3 hours, washed three times with 2 ml of PBS containing 0.05% Tween 20 (PBS-Tween); 50 µl of immunoadsorbent-purified antihuman IgE labeled with <sup>125</sup>I (Kabi-Pharmacia) were added to each tube. The tubes were left overnight at room temperature, washed three times with 2 ml of PBS-Tween, and counted. Results were expressed as B/T (percent). Inhibition by succinylcholine (554 nmol added to 50 µl of serum) was systematically performed for the sera probed positive with the PAPPC assay.

Phadebas RAST alcuronium and Phadebas RAST succinylcholine were performed according to the manufacturer's instructions. QAS RIA with sera from the patient group was performed by Dr. Guéant (INSERM U-308, School of Medicine, Nancy, France) according to the previously published protocol.<sup>7</sup>

## RESULTS

Clinical data and the results of the PAPPC, morphine, TMA, and TEA RIAs for patients who experienced life-threatening reactions to NMBDs are summarized in Table I.

The RIA performed with PAPPC-agarose elicited a low level of binding of <sup>125</sup>I-labeled antihuman IgE for nonallergic, normal sera (mean,  $0.41 \pm 0.19$ ;  $N = 34$ ) and for sera from subjects clinically allergic to drugs other than NMBDs (mean of allergic control group,  $0.31 \pm 0.14$ ;  $N = 35$ ). In contrast, radioactive uptake of antihuman IgE antibodies in sera of patients allergic to NMBDs was significantly higher (mean,  $13.36 \pm 14.92$ ;  $N = 31$ ). A serum was considered positive when the uptake of <sup>125</sup>I-labeled antihuman IgE was higher than the value obtained for normal sera, +3 SD (1% for the PAPPC assay).

The uptake of anti-IgE antibodies for normal sera on morphine-, TMA-, and TEA-Sepharose were, respectively,  $0.29 \pm 0.26$  ( $N = 20$ ),  $0.11 \pm 0.26$  ( $N = 20$ ), and  $0.07 \pm 0.18$  ( $N = 20$ ). A result was considered positive when values were >1.1% for the morphine support, 0.9% for TMA-Sepharose, and 0.6% for TEA-Sepharose. The upper limit of normal sera was 2.3% for the QAS RIA, according to Guéant et al.,<sup>8</sup> and 0.35 PRU/ml for the RAST succinylcholine and the RAST alcuronium, according to the manufacturer's instructions.

As illustrated in Table II, 30 of the 31 patients' sera (97% of the patients) probed positive with the PAPPC

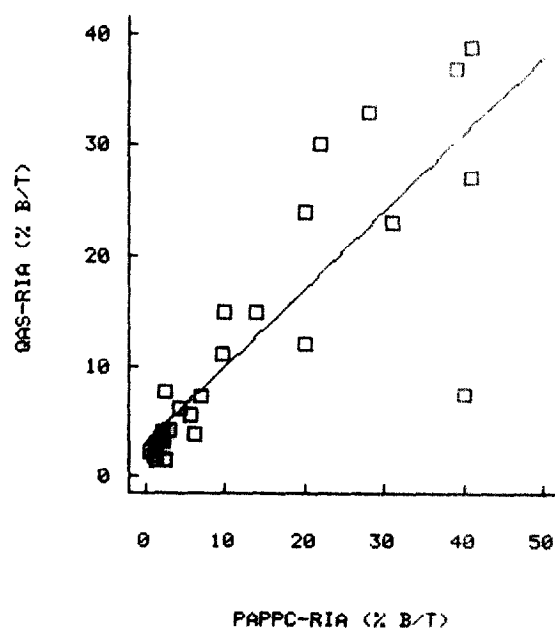


**FIG. 2.** Comparison between PAPPC RIA and QAS RIA in 28 cases of anaphylaxis to NMBDs. Significant linear correlation was observed ( $r = 0.84$ ;  $Y = 2.9 + 0.70 X$ ).

assay. When the RIA was performed with morphine-Sepharose, 25 of the 30 sera tested (83%) elicited a significant uptake of <sup>125</sup>I-labeled antihuman IgE; 94% of the patients allergic to muscle relaxants (29 of the 31 sera) reacted positively in TMA assay, whereas only 60% (18 of 30 sera) of the patients reacted significantly in the TEA assay; 86% of the patients gave a significant uptake of <sup>125</sup>I-labeled antihuman IgE antibodies with the QAS RIA, whereas 70% of the patients were considered as positive with the RAST succinylcholine and only 39% with the RAST alcuronium (data not presented). A significant linear correlation was observed between QAS RIA and PAPPC RIA ( $r = 0.84$ ;  $N = 28$ ;  $y = 2.9 + 0.70 x$ ; Fig. 2).

It should be noted that none of the patients allergic to gallamine reacted with the TEA support. However, there was no significant statistical difference between the uptake of antihuman IgE antibodies on the different supports and the muscle relaxant that induced allergy, except with TEA-Sepharose assay between patients allergic to pancuronium and vecuronium and patients reacting with alcuronium ( $p < 0.05$ ;  $\chi^2 = 3.89$  with Yates' correction).

In the control group, two of the 71 sera (2.8%) were found to be positive with the PAPPC and the TMA assays. A low but significant uptake of antihuman IgE was obtained with the PAPPC assay (1.2% for serum Pi and 1.7% for serum Ja) and with the TMA support (1.2% for serum Pi and 1.1% for serum Ja). The incubation of the two sera with 554 nmol

**TABLE I.** IgE antibodies reactive with PAPPC, morphine, TMA, and TEA in sera from patients who experienced life-threatening anaphylactic reactions to NMBDs

| Patients  | Drug eliciting adverse reactions | Total IgE (kU/L) | Radioactive uptake (%) of <sup>125</sup> I-labeled antihuman IgE and score test (+) or (-) |                         |                           |                         |
|-----------|----------------------------------|------------------|--------------------------------------------------------------------------------------------|-------------------------|---------------------------|-------------------------|
|           |                                  |                  | PAPPC                                                                                      | Morphine                | TMA                       | TEA                     |
| Control   |                                  |                  |                                                                                            |                         |                           |                         |
| Allergic* |                                  |                  | 0.31 ± 0.14†<br>(N = 35)                                                                   | 0.24 ± 0.17<br>(N = 16) | 0.012 ± 0.015<br>(N = 17) | 0.15 ± 0.25<br>(N = 14) |
| Normal‡   |                                  |                  | 0.41 ± 0.19<br>(N = 34)                                                                    | 0.29 ± 0.26<br>(N = 20) | 0.11 ± 0.26<br>(N = 20)   | 0.07 ± 0.18<br>(N = 20) |
| A. L.     | S                                | 47               | 1.5 (+)                                                                                    | 0.6 (-)                 | 1.6 (+)                   | 0.0 (-)                 |
| A. L.     | P                                | 127              | 0.6 (-)                                                                                    | 4.7 (+)                 | 1.9 (+)                   | 3.9 (+)                 |
| B. E.     | P                                | ND               | 36 (+)                                                                                     | 32 (+)                  | 22 (+)                    | 20 (+)                  |
| B. L.     | AL                               | 163              | 5.7 (+)                                                                                    | 5.0 (+)                 | 5.6 (+)                   | 0.2 (-)                 |
| B. O.     | V                                | 690              | 39 (+)                                                                                     | 40 (+)                  | 39 (+)                    | 8.5 (+)                 |
| B. R.     | AL                               | ND               | 7.1 (+)                                                                                    | 7.2 (+)                 | 3.0 (+)                   | 0.3 (-)                 |
| B. R.     | S                                | 282              | 31 (+)                                                                                     | 15 (+)                  | 21 (+)                    | 1.3 (+)                 |
| C. A.     | AL                               | ND               | 2.1 (+)                                                                                    | 2.0 (+)                 | 0.9 (+)                   | 0.0 (-)                 |
| C. L.     | S                                | 65               | 2.4 (+)                                                                                    | 0.2 (-)                 | 0.7 (-)                   | 0.7 (+)                 |
| C. O.     | G/S                              | ND               | 3.0 (+)                                                                                    | 9.0 (+)                 | 6.0 (+)                   | 0.0 (-)                 |
| D. E.     | V                                | ND               | 1.4 (+)                                                                                    | 0.5 (-)                 | 1.0 (+)                   | 0.6 (+)                 |
| E. L.     | S                                | 853              | 14 (+)                                                                                     | 13 (+)                  | 17 (+)                    | 1.4 (+)                 |
| F. A.     | V                                | 212              | 22 (+)                                                                                     | 30 (+)                  | 25 (+)                    | 7.9 (+)                 |
| F. A.     | G/S                              | ND               | 1.2 (+)                                                                                    | 0.8 (-)                 | 0.8 (-)                   | 0.0 (-)                 |
| F. I.     | AL                               | ND               | 20 (+)                                                                                     | 19 (+)                  | 15 (+)                    | 2.8 (+)                 |
| G. A.     | V                                | 26               | 6.1 (+)                                                                                    | 3.2 (+)                 | 2.8 (+)                   | 1.9 (+)                 |
| G. R.     | V                                | ND               | 20 (+)                                                                                     | 31 (+)                  | 24 (+)                    | 5.6 (+)                 |
| L. E.     | V                                | 50               | 2.9 (+)                                                                                    | 2.8 (+)                 | 2.8 (+)                   | 0.0 (-)                 |
| M. A.     | AL                               | ND               | 9.6 (+)                                                                                    | 16 (+)                  | 9.4 (+)                   | 0.0 (-)                 |
| M. E.     | AT                               | 123              | 10 (+)                                                                                     | 13 (+)                  | 13 (+)                    | 1.7 (+)                 |
| M. U.     | G                                | ND               | 1.8 (+)                                                                                    | 3.1 (+)                 | 3.6 (+)                   | 0.0 (-)                 |
| N. A.     | S                                | 35               | 2.3 (+)                                                                                    | 0.6 (-)                 | 2.0 (+)                   | 0.0 (-)                 |
| N. O.     | G                                | 118              | 2.4 (+)                                                                                    | ND                      | 1.5 (+)                   | ND                      |
| O. D.     | P                                | 244              | 7.1 (+)                                                                                    | 8.5 (+)                 | 6.9 (+)                   | 6.8 (+)                 |
| P. L.     | P                                | ND               | 40 (+)                                                                                     | 29 (+)                  | 16 (+)                    | 0.7 (+)                 |
| R. A.     | S                                | 425              | 41 (+)                                                                                     | 42 (+)                  | 35 (+)                    | 28 (+)                  |
| R. E.     | AL                               | ND               | 28 (+)                                                                                     | 12 (+)                  | 21 (+)                    | 4.2 (+)                 |
| S. I.     | AL                               | ND               | 41 (+)                                                                                     | 44 (+)                  | 31 (+)                    | 0.8 (+)                 |
| S. T.     | V                                | 300              | 1.8 (+)                                                                                    | 1.2 (+)                 | 2.6 (+)                   | 2.4 (+)                 |
| T. O.     | S                                | 23               | 2.1 (+)                                                                                    | 1.4 (+)                 | 2.6 (+)                   | 0.4 (-)                 |
| V. A.     | AL                               | 417              | 1.7 (+)                                                                                    | 2.4 (+)                 | 0.9 (+)                   | 0.0 (-)                 |

S, Succinylcholine P, pancuronium; AL, alcuronium; V, vecuronium; G, gallamine; AT, atracurium; ND, not determined.

\*Thirty-five allergic subjects who reacted clinically to drugs other than NMBDs (23 female and 12 male subjects).

‡Thirty-four normal subjects (30 female and four male subjects).

†Mean ± SD.

of succinylcholine before the PAPPC assay induces 83% of inhibition of <sup>125</sup>I-labeled anti-IgE uptake for the serum Ja and 60% for the serum Pi, confirming the specificity of the IgE detected.

A subject from the patients' group, allergic to NMBDs, was found to be negative with PAPPC assay but positive with morphine-, TMA-, and TEA-Sepharose assays. The specificity of the IgE detected with the morphine support was confirmed by a sig-

nificant inhibition with pancuronium, which induced anaphylaxis, and vecuronium.

Total IgE serum level and the time period between anaphylaxis and the blood tests are not correlated with the PAPPC RIA results ( $r = 0.558$ ;  $N = 16$ ; and  $r = 0.014$ ;  $N = 30$ , respectively). No significant correlation coefficient was found between these two parameters and the uptake of anti-IgE obtained on morphine-, TMA-, and TEA-Sepharose. Thus, these

**TABLE II.** Presence of serum IgE antibodies to different solid phases containing tertiary or quaternary ammonium ions in subjects who reacted to alcuronium, succinylcholine, gallamine, pancuronium, vecuronium, and atracurium

| No. of subjects            | Drugs reacted to clinically | IgE antibodies detected with solid phases of |              |            |             |
|----------------------------|-----------------------------|----------------------------------------------|--------------|------------|-------------|
|                            |                             | PAPPC (%)                                    | Morphine (%) | TMA (%)    | TEA (%)     |
| 8                          | Alcuronium                  | 8 (100)                                      | 8 (100)      | 8 (100)    | 3 (38)      |
| 7                          | Succinylcholine             | 7 (100)                                      | 4 (57)       | 6 (86)     | 4 (57)      |
| 4                          | Gallamine                   | 4 (100)                                      | 2 on 3* (66) | 3 (75)     | 0 on 3* (0) |
| 4                          | Pancuronium                 | 3 (75)                                       | 4 (100)      | 4 (100)    | 4 (100)     |
| 7                          | Vecuronium                  | 7 (100)                                      | 6 (86)       | 7 (100)    | 6 (86)      |
| 1                          | Atracurium                  | 1 (100)                                      | 1 (100)      | 1 (100)    | 1 (100)     |
| No. and % positive overall |                             | 30/31 (97)                                   | 25/30 (83)   | 29/31 (94) | 18/30 (60)  |

\*Only three sera of the four from patients who reacted clinically to gallamine were available in sufficient amount to perform morphine and TEA.

two parameters do not interfere with either the PAPPC assay or with the three other RIAs.

The uptake of  $^{125}\text{I}$ -labeled antihuman IgE antibodies on PAPPC support was inhibited by succinylcholine in all the sera found positive by PAPPC assay (data not presented). Typical inhibition curves of two sera with alcuronium, atracurium, pancuronium, succinylcholine, and vecuronium are illustrated in Fig. 3.

The inhibition experiments confirmed the cross-reactivity of the different NMBDs in the in vitro tests.

## DISCUSSION

The detection rate of IgE antibodies with the PAPPC RIA (97%) was higher than that obtained for other RIAs with drug solid-phase complexes, such as TMA (94%), QAS (86%), morphine (83%), and TEA (60%), and for the Phadebas RAST succinylcholine (70%) and Phadebas RAST alcuronium (39%). Furthermore, the specificity and the positive predictive value of the PAPPC RIA were also high (97% and 94%, respectively).

The sensitivity of QAS RIA (86%) with 28 patients included in this study is similar to the value previously reported for this test (87%).<sup>8</sup> Our results with TEA and morphine assays are also in the range found by Harle et al.<sup>7</sup> for these tests: 42% to 58% for the TEA assay and 44% to 83% with the morphine RIA, depending on the muscle relaxant involved in anaphylaxis. In our study, by using the two assays, PAPPC and TMA elicited 100% sensitivity, whereas Harle et al.<sup>7</sup> needed three assays with different supports to reach this score.

Two subjects of the control group were considered positive with the PAPPC and the TMA assays, even though intradermal tests with muscle relaxants were

negative in both cases. It should be noted that they received NMBDs and the inhibition experiments probed the specificity of the detected IgE antibodies. According to the method of Didier et al.,<sup>9</sup> the presence of IgE antibodies to the allergic determinants does not appear sufficient to induce allergic reactions. These authors have demonstrated that pancuronium could bind specific IgE antibodies in sera, although it might not be able to trigger mast cells, eliciting negative skin tests. These two positive sera did not induce a low positive predictive value (94%).

The serum from the patients' group that was negative with PAPPC assay was also negative with the QAS RIA and did not appear to contain IgE antibodies directed to quaternary ammonium.

Serum IgE level and/or the delay between anaphylaxis and blood tests do not interfere with the PAPPC assay or with the morphine, TMA, and TEA RIAs. In the same way, no significant correlation has been found between serum IgE level and the results of QAS RIA by other investigators.<sup>10</sup>

We obtained a far higher sensitivity for the TMA assay than for the TEA assay. Furthermore, all sera except one of the 31 sera tested in this study revealed a greater radioactive uptake of  $^{125}\text{I}$ -labeled antihuman IgE antibodies with TMA-Sepharose than with TEA-Sepharose. These results differ from results of Harle et al.<sup>7</sup> who observed a greater radioactive uptake of  $^{125}\text{I}$ -labeled antihuman IgE antibodies with TEA-Sepharose than with TMA support, at least when succinylcholine, alcuronium and/or D-tubocurarine were involved in anaphylaxis, and concluded that TEA-Sepharose was a more suitable support for the detection of IgE antibodies reactive with tertiary ammonium groups than TMA-Sepharose.

The differences between our results and results of

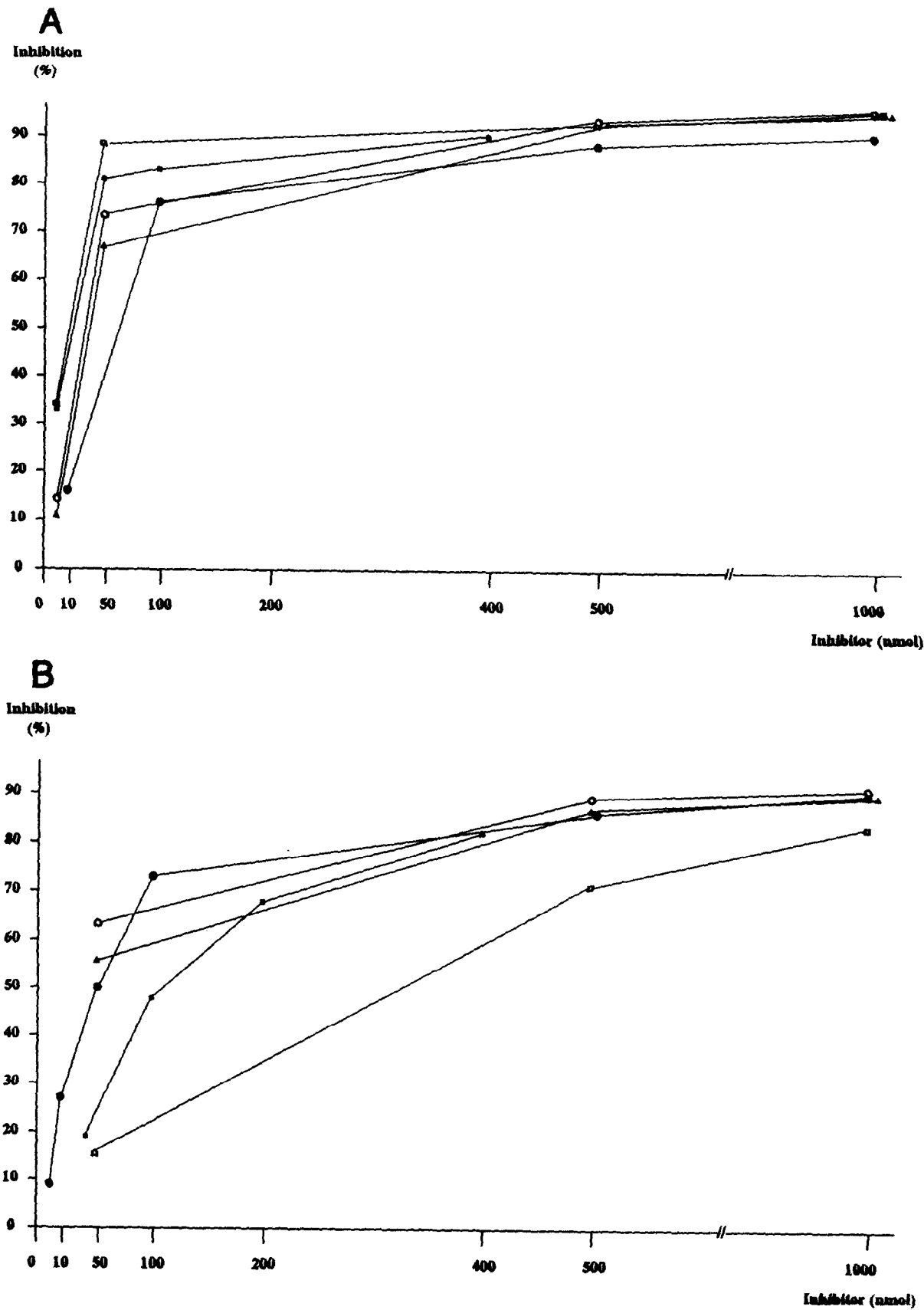


FIG. 3. Inhibition by a number of NMBDs of IgE binding, **A**, in serum E1, and **B**, in serum Ga to PAPPC agarose. Serum E1 was used at a dilution of 1:4; serum Ga was used undiluted; succinylcholine (▲), alcuronium (□), atracurium besylate (■), pancuronium (○) and vecuronium (●).

Harle et al.<sup>7</sup> might arise from several parameters. First, the time period between the blood test and the anaphylactic-like reactions was relatively short (1 month) and homogeneous for all the patients in their study, whereas it was markedly longer and variable in the present study (1 to 10 months for 18 patients, and 2 to 15 years in 13 other cases). Some selected subpopulations of IgE directed against determined epitopes of muscle relaxant molecules might disappear with time, whereas other subpopulations persist. Second, 100% of anaphylactoid reactions induced by vecuronium<sup>7</sup> and by pancuronium<sup>4</sup> were detected with the TMA assay in this study. However, these NMBDs were not among the drugs eliciting reactions in the sera assayed by Harle et al.,<sup>7</sup> and that difference might explain, at least in part, the difference we observed in sensitivity.

PAPPC, like choline hydrochloride used in QAS RIA, contains a quaternary ammonium ion and TMA, a tertiary ammonium group. In these three compounds, the nitrogen atom is substituted by three methyl groups. TEA contains ethyl groups instead of methyl groups. Since the frequency of detection of IgE antibodies is higher with the solid phases containing a nitrogen atom substituted by three methyl groups (PAPPC, TMA, and QAS) than that with TEA-Sepharose, it appears that the presence of the methyl groups as substituents of the ammonium (tertiary or quaternary) immobilized on the solid phase increases the frequency of detection of IgE antibodies reactive to NMBDs. It is difficult to explain the difference in sensitivity among the three RIAs with choline derivatives. It should be noted that the two less sensitive RIAs (QAS RIA and Phadebas RAST succinylcholine) are performed with solid-phase complexes prepared with simple choline derivatives (choline hydrochloride and choline bicarbonate, respectively), whereas PAPPC agarose, eliciting the highest sensitivity, is prepared with a larger choline derivative, including an aromatic ring and a phosphate group.

In conclusion, this new RIA with PAPPC appears to be the most efficient test in screening sera for the presence of IgE antibodies, whatever the muscle relaxant involved in anaphylaxis. One important ad-

vantage is that the solid-phase PAPPC is commercially available and ready to use. This advantage will improve accuracy for the comparison of the results from different laboratories by avoiding the irreproducibility caused by the coupling procedures.

The possibility of or to use the PAPPC RIA as a predictive test would be very helpful to prevent fatal reaction to NMBDs during GA for patients at risk<sup>11</sup> and is currently under investigation.

We thank Mrs. C. Juillet for excellent technical assistance.

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