

# Effect of guinea-pig purified immunoglobulin G1 on the responsiveness of tracheal, aortic, vas deferens and ileum smooth muscles

J.-C. BIDON, A. T. VU, M. BLIN, M. GOGNY, O. SOUILEM and A. JONDET

Laboratoire de Physiologie et Pharmacodynamie, Institut National de la Recherche Agronomique, Ecole Nationale Vétérinaire, Nantes, France

## Summary

**Background** Smooth muscles hyperresponsiveness is a common feature in anaphylaxis and allergic diseases.

**Objective** The aim of the present work was to investigate the effect of *in vitro* passive sensitization with highly purified immunoglobulin G1 (IgG1) on the responsiveness of tracheal, aortic, vas deferens and ileum smooth muscles.

**Methods** Firstly, IgG1, obtained from actively sensitized BFA guinea-pigs, was purified by Protein A-Sepharose column and characterized by enzyme-linked immunosorbent assay (ELISA) and immunoelectrophoresis analysis. Concentration-response curves to spasmogens (acetylcholine for trachea and vas deferens, noradrenaline for aorta and histamine for ileum) were established before and after *in vitro* passive sensitization with IgG1.

**Results** Contractile responses and maximal contractions were significantly enhanced after passive sensitization for all the organs. Maximal contractions were significantly increased in the trachea (+46.7%), aorta (+51%), vas deferens (+114.2%) and ileum (+117.2%). At the end of the experiments, the application of the sensitizing antigen induced a significant Schultz–Dale reaction of the smooth muscles.

**Conclusion** The present results show that the *in vitro* application of purified IgG1 can produce non-specific smooth muscle hyperreactivity and hypersensitivity. So, IgG1 can be considered as the main factor involved in the genesis of sensitization-induced hyperresponsiveness, and probably play a great role in hyperreactivity observed during allergic diseases and anaphylaxis.

**Keywords:** immunoglobulin G1, hyperresponsiveness, hyperreactivity, sensitization, purification, smooth muscle, trachea, aorta, vas deferens, ileum

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## Introduction

We recently reported that the active sensitization of guinea-pigs *in vivo* to egg albumen led to contractile hyperresponsiveness in various muscles [1,2]. In this

and earlier studies [1–6] active sensitization induced hyperresponsiveness in trachea, bronchus, vas deferens, ileum and aorta [7]. Consequently, a common defect may occur after sensitization in these different muscles.

In guinea-pigs, immunoglobulin G1 (IgG1) has already been recognized as a reaginic antibody responsible for the mediation of the antigen-induced contraction in actively and passively sensitized trachea [8–11]. Furthermore, *in vitro* passive sensitization with IgG1-induced

Correspondence: Dr J.-C. Bidon, Laboratoire de Physiologie et Pharmacodynamie, INRA, Ecole Nationale Vétérinaire, Route de Gachet, C.P. 3013, F-44087 Nantes, France.

alterations in the resting membrane potential of the airway smooth muscle cells [11–14].

The aim of the work was to purify and to characterize IgG1 from actively sensitized guinea-pigs and to investigate whether *in vitro* passive sensitization to highly purified IgG1-induced hyperresponsiveness to spasmogens in trachea, aorta, vas deferens and ileum.

## Materials and methods

### Animals

Male guinea-pigs (weighing 250–350 g) from the BFA strain (Bertho, Dinan, France) were used.

### Preparation of sensitized serum

Guinea-pigs were actively sensitized as previously described [1,2]. Briefly, active sensitization was performed by one subcutaneous injection/day for 5 days of 0.5 mL of egg albumen dissolved in 1 v/v bidistilled water (26 µg albumen protein/day). The animals were bled 22–37 days after the first injection, and the sensitized serum was stored at –30°C until analysis.

### Separation of IgG1 and IgG2 antibodies by affinity chromatography

The IgG1 and IgG2 anti egg albumen were separated by Protein A-Sepharose column chromatography as previously described [15]. The antiserum to be separated was desalted through a column of sephadex G 25 M with citrate phosphate buffer, pH 7.3 and chromatographed on a Protein A-Sepharose CL-4B which had been equilibrated with the same buffer. Citrate phosphate buffers were prepared by adding 0.1 M citric acid to 0.2 M Na<sub>2</sub>HPO<sub>4</sub> and the pH was decreased by further addition of 0.1 M citric acid. The bound antiserum was eluted from the column with citrate phosphate buffers of decreasing pH (6.5, 6.0, 5.5, 5, 4.7, 4.5, 4.3, 3.2, 2.6) respectively. The antisera fractions were buffered immediately by 1 M Tris to pH 7.4 and were calculated from the absorbance at 280 nm used to determine the protein concentration.

### Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The SDS-PAGE was performed according to Laemmli [16] in reducing conditions with β mercaptoethanol on a slab gel with 12% acrylamide in separating gel and 4% acrylamide in stacking gel. After electrophoresis, gels were stained with Coomassie blue R 250.

### Immunochemical techniques for IgG1 specificity

Immunoelectrophoresis was performed according to Scheidegger [17] in 1% agarose gel (Agarose A 45-IBF) on glass plates in the veronal buffer at pH 8.4. Wells were cut in the gel and filled with guinea-pig Protein A-Sepharose fractions (concentration of 1 mg/mL). Electrophoresis was performed for 2 h. The rabbit anti-guinea-pig IgG1 or IgG2 were placed in the trough. The diffusion time was usually 1 or 2 days. Plates were stained as before.

The enzyme linked immunoassay (ELISA) used for measurement of IgG1 in guinea-pigs has been described in detail previously by Tijssen [18]. The flat-bottomed microtitre plates NUNC (Roskilde, Denmark) were coated with the fraction purified by Protein A-Sepharose (1 µg/mL) diluted in a bicarbonate buffer (pH 9.6). After incubation for 18 h at 4°C, the plates were washed with PBS Tween three times. Next, the fraction purified-coated plates were treated with dilutions of rabbit antiserum (1:1000) in PBS Tween and incubated for 1 h at 37°C. After plates were again washed, they were finally incubated for 1 h at 37°C with goat anti rabbit IgG (heavy and light chain specific) coupled peroxidase (1:2000) in PBS Tween. Plates were washed again, and the substrate solution (*O*-Phenyldiamine and H<sub>2</sub>O<sub>2</sub>) was added. After 30 min, the reaction was stopped with H<sub>2</sub>SO<sub>4</sub> and the optical densities were read at 492 nm with an ELISA reader.

All chemicals were obtained from Biorad (France) and were of analytical grade, except the low molecular weight and protein A sepharose CL-4B from Pharmacia (France). Monospecific antisera against guinea-pig IgG, IgG1 and IgG2 were obtained from Jackson Immuno Research (Westgrove, PA, USA).

### Experimental procedure

Isolated tissues were prepared as previously described [1,2,19]. Briefly, non-sensitized guinea-pigs fasted overnight were killed by a blow on the head and exsanguinated. The trachea, the aorta, the right vas deferens and the ileum were excised and placed in a separate Petri dish containing the appropriate physiological salt solution (see composition below). Surrounding connective tissues and blood vessels were removed from the trachea. A cartilaginous ring was prepared at 1 cm from the larynx and suspended under optimal resting tension of 1 g in a bath (volume of 5 mL) containing modified Krebs-Henseleit solution (mM: NaCl, 118; KCl, 4.7; CaCl<sub>2</sub>, 2.52; MgCl<sub>2</sub>, 0.25; NaH<sub>2</sub>PO<sub>4</sub>, 1; NaHCO<sub>3</sub>, 24.88; Glucose, 11.1) equilibrated with carbogen (95% O<sub>2</sub> + 5% CO<sub>2</sub>) and maintained at 35°C. The aorta was removed

near the heart, freed of excess adherent connective tissues and cut into rings of 2 mm wide. Then, the rings were suspended under 1 g resting tension in an organ bath containing modified Van Newton solution (mM: NaCl, 133.4; KCl, 5.6; CaCl<sub>2</sub>, 3.6; MgCl<sub>2</sub>, 1.04; NaH<sub>2</sub>PO<sub>4</sub>, 0.4; NaHCO<sub>3</sub>, 11.9; Glucose, 5.55) equilibrated with carbogen and maintained at 38°C. The vas deferens was freed of excess adherent connective and adipose tissues and then suspended under 3 g resting tension in an organ bath containing modified Krebs-Henseleit solution equilibrated with carbogen and maintained at 37°C. A longitudinal strip of the terminal portion of ileum was removed, prepared free of excess tissue, rubbed to eliminate mucosal surface and suspended under 2 g resting tension in an organ bath containing Tyrode solution (mM: NaCl, 136.9; KCl, 2.68; CaCl<sub>2</sub>, 1.8; MgCl<sub>2</sub>, 0.49; NaH<sub>2</sub>PO<sub>4</sub>, 0.42; NaHCO<sub>3</sub>, 11.9; Glucose, 5.5) equilibrated with carbogen and maintained at 36°C.

The drugs used were: acetylcholine chloride (Sigma), histamine dihydrochloride (Sigma) and noradrenaline bitartrate (Sigma). Drugs were prepared on the day of the experiment as a 1 mM stock solution in bidistilled water and diluted with the appropriate control medium to obtain the required final concentrations.

#### *Isometric measurement of smooth muscle reactivity*

Contractile responses were recorded as previously described by Souilem *et al.* [19]. Smooth muscle strips were attached at one end to a force isometric transducer using an inductive proximity sensor (Electromike from Electrocorporation, Sarasota, FLA, USA) and submitted to a basal resting tension (see above). The other end of the preparation was fixed to the organ bath. Preparations were allowed to equilibrate in control medium for a period of 1 h for trachea, aorta and vas deferens, and 3 h for ileum. During that period, the preparations were washed every 15 min for trachea, vas deferens and aorta and 30 min for ileum. Isometric tension was recorded on a potentiometric recorder (Bryans-BS 272, USA) and expressed in mN. For each organ, a submaximal dose of the drug was firstly applied after the period of equilibration. After that, a first concentration-response curve (CRC) was established by the single dose method (every 15 min for the trachea, 5 min for the vas deferens and ileum) and in a cumulative manner for the aorta (every 30 min). Passive *in vitro* sensitization with purified IgG1 was then performed in the organ bath. After removal of IgG1 from the bath and an equilibration period of 15 min for trachea, vas deferens and ileum, and 30 min for aorta, a second CRC was constructed. *In vitro* challenge with the sensitizing antigen (0.1 mL) was finally performed to

test the antigen contractile capacity (Schutz-Dale reaction [20]). The contractile responses were measured at the peak contraction.

#### *Passive in vitro sensitization*

Passive *in vitro* sensitization of smooth muscle preparations from trachea, vas deferens, aorta and ileum was performed using a modified technique from Regal [9]. Briefly, each organ isolated from normal nonsensitized animals were incubated with highly purified IgG1 (100 µg in the organ bath of 5 mL) for 45 min at 35, 36, 37 and 38°C according to the organ (see above). The dose of IgG1 for incubation and the period of it have been determined to be the most effective in our experimental procedure. IgG1 was removed by washing three times minimum within 15 min the organ bath at the end of the incubation.

#### *Data analysis and statistics*

All data are expressed as mean  $\pm$  SEM. Total CRC were statistically evaluated using the two-way analysis of variance test (ANOVA) with repeated measures, and all pairs of points were then compared by the contrast method. In another hand, a computerized curve-fitting technique based on the four parameter logistic equation (previously described in Souilem *et al.* 1992 [19]) analysed the CRC of the preparation [21,22]. Specific curve parameters were then determined (amplitude of the maximal contraction: E<sub>m</sub>; pD<sub>2</sub> defined as  $-\log EC_{50}$ ; slope factor: S) and characterized the shape of the CRC. Levels of significance between these calculated parameters obtained from control and sensitized CRC were calculated using the paired Student's *t*-test. A value of  $P < 0.05$  or less was considered to be significant.

## **Results**

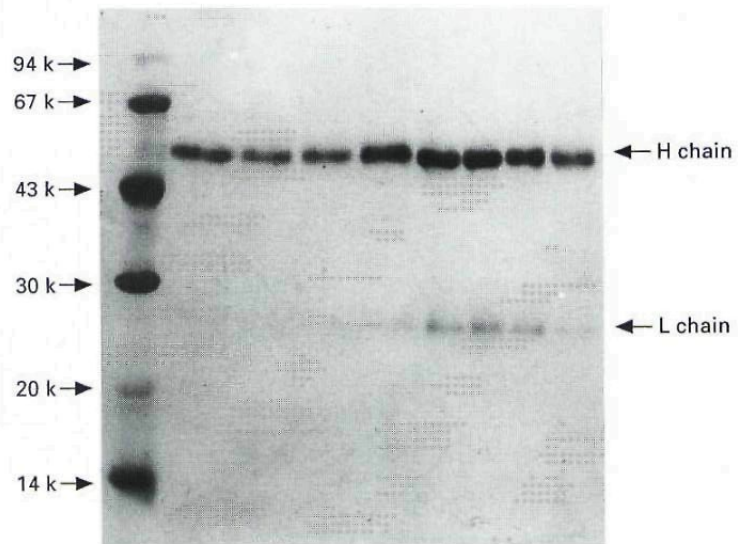
#### *Affinity chromatography and SDS-PAGE*

The IgG1 eluted from the Protein A-Sepharose column using a pH gradient of 6.5 was subjected to SDS-PAGE under reducing conditions. The resulting gel (Fig. 1) demonstrated that the eluted materials resolved in two bands whose respective molecular weights could be estimated by a standard run with proteins known molecular weight. The IgG1 heavy chain appeared as one band about 53 kDa and one band at 23 kDa that was attributed to the light chain.

#### *Immunochemical identification of IgG1*

The guinea-pig has been found to possess two IgG

**Fig. 1.** Affinity chromatographic purification of guinea-pig IgG1-SDS polyacrylamide gel electrophoresis under reducing conditions with Coomassie blue stain. Lane 1. Molecular weight standards. Lanes 2–9. Guinea-pig IgG1 eluted from the chromatography column; note heavy (gamma) and light chain bands.



subclasses, IgG1 and IgG2, differing in many characteristics, particularly in their immunoelectrophoretic mobility [23]. In fact, immunoelectrophoretic analysis (Fig. 2a) revealed that the IgG1 resulted in a strong precipitation line of identity against rabbit anti guinea-pig IgG1. No line was observed against purified IgG2 (pH 4.7–4.3). The IgG1 had fast  $\gamma$  immunoelectrophoretic mobility. On the contrary, the IgG2 fractions had slow  $\gamma$  mobility (Fig. 2b). The IgG1 fraction was determined by indirect ELISA with rabbit antisera. The IgG2 was not significantly contaminated with IgG1. Using the ELISA, it was possible to detect as little as 10 ng/mL the IgG1 guinea-pig fraction.

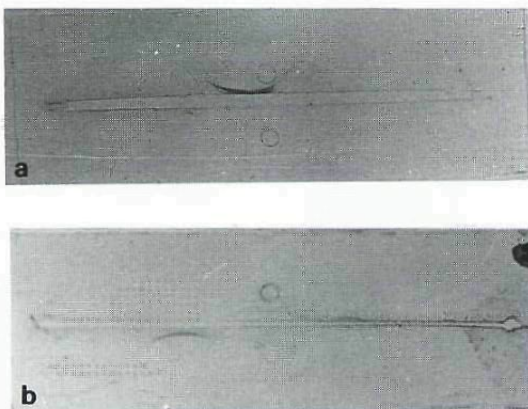


**Fig. 2.** Immunoelectrophoretic analysis of guinea-pig IgG1 and IgG2 eluted from the affinity chromatography. a. Wells were filled with purified guinea-pig IgG1 and IgG2, trough was filled with rabbit anti guinea-pig IgG1. b. Wells were filled with purified guinea-pig IgG1 and IgG2, trough was filled with rabbit anti guinea-pig IgG2.

#### Smooth muscle reactivity

Spasmogens elicited a dose-dependent CRC in trachea, aorta, vas deferens and ileum. *In vitro* passive sensitization with highly purified IgG1 induced significant upward shifts of the CRCs to acetylcholine in trachea and vas deferens ( $P < 0.05$  for both by ANOVA, Fig. 3), to noradrenaline in aorta ( $P < 0.001$  by ANOVA, Fig. 3) and to histamine in ileum ( $P < 0.05$  by ANOVA, Fig. 3). These shifts were dose-dependent as the interactions, determined by ANOVA between spasmogens concentration and sensitization, were significant ( $P < 0.01$  for trachea and vas deferens,  $P < 0.001$  for aorta and ileum). Furthermore, using the contrast method, significant differences between tracheal contractions developed before and after sensitization were found from  $10^{-6}$  to  $10^{-3}$  M acetylcholine ( $P < 0.01$ ). Responsiveness of sensitized aorta was significantly increased for the highest noradrenaline concentrations ( $P < 0.05$  for  $10^{-6}$  M and  $P < 0.001$  between  $10^{-5}$  and  $10^{-3.5}$  M). In the vas deferens, sensitization-induced hyperresponsiveness was dose-dependent and significant differences between contractions from control and sensitized preparations were only observed for  $10^{-4}$  and  $10^{-3}$  M acetylcholine ( $P < 0.01$ ). Significant differences in ileum contractions were detected after sensitization for the highest histamine concentrations ( $10^{-5}$ – $10^{-3}$  M,  $P < 0.01$ ).

The IgG1-induced hyperresponsiveness was characterized by a significant enhancement of the  $E_m$  of the CRCs, although changes in  $EC_{50}$  were only significant for aorta and ileum (Table 1). After passive sensitization,  $E_m$  in response to acetylcholine was significantly increased by  $41.3 \pm 13\%$  and  $147.3 \pm 62.4\%$  in trachea and vas deferens respectively. Furthermore,  $E_m$  for noradrenaline CRC in

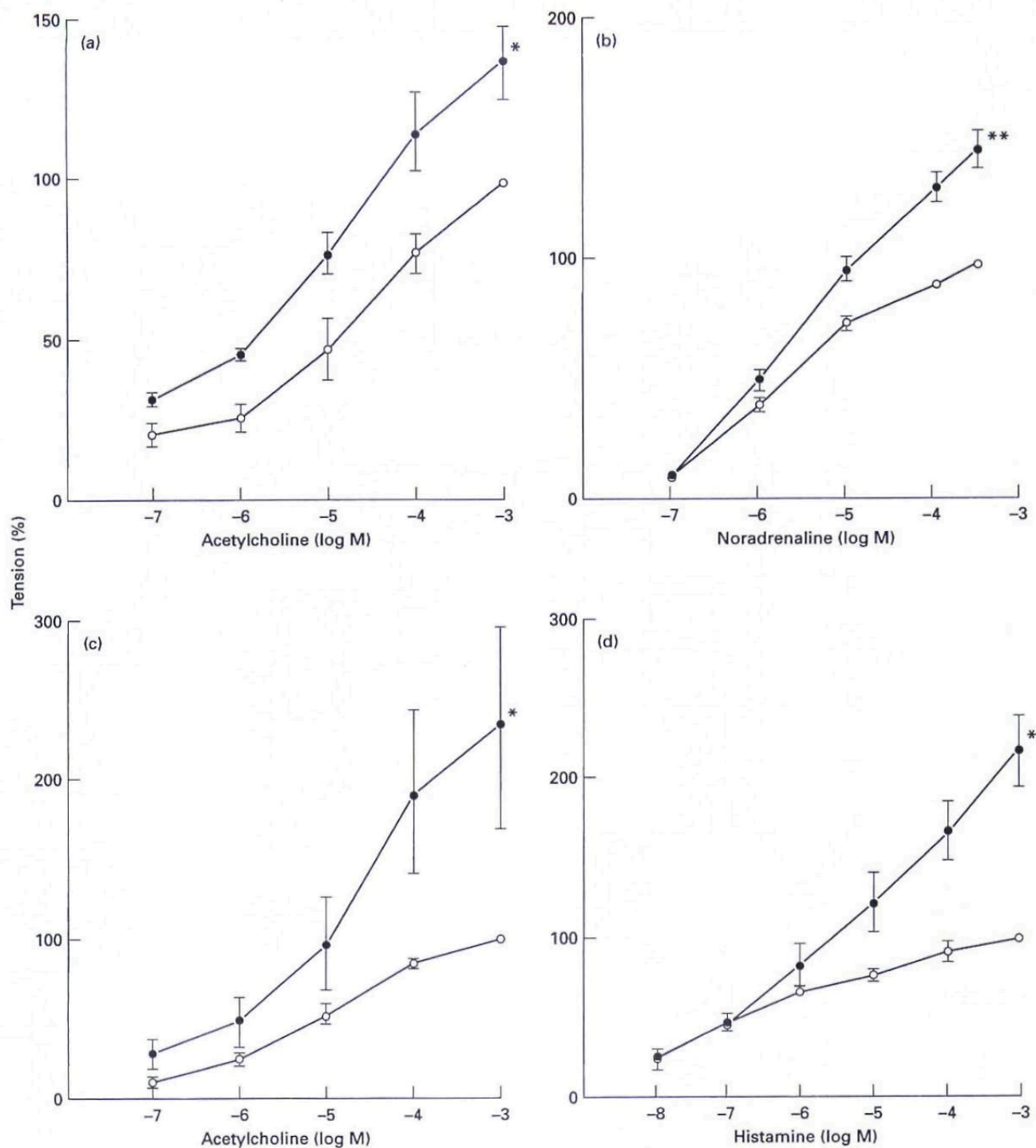


**Fig. 3.** Effect of passive *in vitro* IgG1 sensitization on the contractions of trachea (a), aorta (b), vas deferens (c) and ileum (d) to acetylcholine, noradrenaline, acetylcholine and histamine respectively. Values are presented as the percentage of the control response obtained for the highest concentration of the spasmogen;  $n = 6, 10, 8, 6$  for trachea, aorta, vas deferens and ileum respectively. \* $P < 0.05$ , \*\* $P < 0.001$ : significant difference from the control determined by ANOVA.

**Table 1.** Characteristics of concentration-response curves to acetylcholine in trachea and vas deferens, noradrenaline in aorta, and histamine in ileum obtained from control and IgG1-sensitized preparations

|                                | Control        |               |               | Sensitized          |                    |               |
|--------------------------------|----------------|---------------|---------------|---------------------|--------------------|---------------|
|                                | $E_m$          | pD2           | S             | $E_m$               | pD2                | S             |
| Trachea (6) Acetylcholine      | $11.4 \pm 1.4$ | $4.8 \pm 0.3$ | $0.9 \pm 0.1$ | $16.7 \pm 2.9^*$    | $5.1 \pm 0.2$      | $1.2 \pm 0.1$ |
| Aorta (10) Noradrenaline       | $9.6 \pm 1.4$  | $5.7 \pm 0.1$ | $0.6 \pm 0.0$ | $14.5 \pm 2.1^{**}$ | $5.4 \pm 0.1^{**}$ | $0.7 \pm 0.0$ |
| Vas Deferens (8) Acetylcholine | $43.0 \pm 6.5$ | $5.1 \pm 0.2$ | $0.7 \pm 0.1$ | $92.1 \pm 19.4^*$   | $4.6 \pm 0.1$      | $0.9 \pm 0.2$ |
| Ileum (6) Histamine            | $11.6 \pm 2.6$ | $6.5 \pm 0.3$ | $1.3 \pm 0.2$ | $25.2 \pm 6.9^*$    | $5.3 \pm 0.2^{**}$ | $1.2 \pm 0.1$ |

$E_m$  is the maximal contraction expressed in mN. pD2,  $-\log EC_{50}$  where  $EC_{50}$  is the concentration for half maximal contraction. S is the slope factor. Data are means  $\pm$  SEM. (n) represents the number of experiments. \* $P < 0.05$ , \*\* $P < 0.01$ , significant difference from control.

aorta was significantly enhanced by  $53.2 \pm 8.4\%$  after sensitization and  $E_m$  to histamine was increased by  $118.1 \pm 27.5\%$  in the sensitized ileum (Table 1).

#### *IgG1 sensitization and antigen challenge*

IgG1 sensitization and antigen challenge led to variable results according to the kind of organ. *In vitro* IgG1 incubation for 45 min induced an early contraction in two of 10 aortae, four of six vas deferens and six of six ileum and produced a decrease in basal tone of all the tracheae (Table 2). Three of six tracheae and all vas deferens and ileum contracted upon *in vitro* application of egg albumen (Table 2).

#### **Discussion**

In this study, highly purified IgG1 were prepared from BFA guinea-pigs which were actively sensitized to egg albumin. *In vitro* passive sensitization to IgG1 induced an increase in contractile responses to spasmogen in the trachea, aorta, vas deferens and ileum of the non-sensitized BFA guinea-pig. Most of the previous studies concerning smooth muscle contractile hyperresponsive-

ness after sensitization were carried out on airways from actively sensitized animals [2,4–6,24–26] except recent reports investigating the reactivity of the aorta [7] the ileum and the vas deferens [1].

The experiments were performed such as the same organ strip was firstly used as control and secondly as passively sensitized. Preliminary studies showed no significant difference in contractile responses between first controls and second time-matched non-sensitized preparations. Our results showed an hyperreactivity after *in vitro* passive sensitization with IgG1 that was characterized in the four preparations studied by a significant upward shift of the CRC with a significant increase in  $E_m$ . In the present study, hyperresponsiveness in trachea and vas deferens was present without a change in  $EC_{50}$  whereas an increase in  $EC_{50}$  was observed for aorta and ileum. Previous reports have already demonstrated hyperresponsiveness of the ileum, vas deferens [1] and trachea [2,4] from the guinea-pig after active sensitization to egg albumen.

The characteristics of the hyperresponsiveness suggested intracellular changes in the responding smooth muscle tissue. Furthermore, as contractile hyperresponsiveness produced by active sensitization could be reproduced

**Table 2.** Change in tension during IgG1 incubation and antigen (albumen) application in trachea, aorta, vas deferens and ileum preparations

|         | Trachea              | Aorta                | Vas deferens         | Ileum               |
|---------|----------------------|----------------------|----------------------|---------------------|
| IgG1    | $-0.6 \pm 0.2$ (6/6) | $3.4 \pm 0.2$ (2/10) | $10.1 \pm 2.4$ (4/6) | $4.9 \pm 1.2$ (6/6) |
| Antigen | $1.6 \pm 0.8$ (3/6)  | Not Done             | $12.3 \pm 3.7$ (6/6) | $5.9 \pm 0.3$ (6/6) |

Data are means  $\pm$  SEM (mN); (n/m) represents the proportion of experiments where a response was observed. In the trachea, the decrease in basal tone is indicated as a negative value.

by IgG1 *in vitro* passive sensitization, present results demonstrate that IgG1 is directly involved in the alteration of the smooth muscles reactivity. Indeed, during *in vitro* passive sensitization, when a contractile response of the smooth muscles was observed it can be suggested that IgG1 could activate a contractile pathway. Souhrada and Souhrada [11–14] reported IgG1-induced alterations in the resting membrane potential of the airway smooth muscle cells. IgG1 might bind to smooth muscle cells membranous Fc receptors [14] and then would potentiate contractile responses to spasmogens through an unknown mechanism. In the present study, the preparations were washed many times between passive sensitization and antigen challenge. The contraction produced by the antigen demonstrated the presence of IgG1 bound to the isolate strips. However, we have not investigated here whether IgG1 could be linked to smooth muscle cells or to any mast cells present in the preparations [27].

A possible explanation for the IgG1-induced changes in smooth muscle behaviour has been proposed by Souhrada and Souhrada [11]. IgG1 produced, in a first step, a smooth muscle cell depolarization with a contractile response and, in a second step, an hyperpolarization of the cells. These changes were amiloride-dependent suggesting an interaction of IgG1 with the membranous  $\text{Na}^+/\text{H}^+$  exchange or the sodium channel. Then, IgG1 caused an increase in the sodium influx that could lead to the activation of reverse mode of functioning of the  $\text{Na}^+/\text{Ca}^{++}$  exchange and an enhanced calcium influx in the cell. Increased calcium availability from intracellular origin has been reported by Ortiz *et al.* [5] in the trachea from actively sensitized guinea-pigs and seemed to be mainly involved in the sensitization-induced hyperresponsiveness of smooth muscles to spasmogens [1].

Several other hypotheses had been pointed out to explain sensitization-induced hyperresponsiveness such as epithelium or endothelium damage in trachea and blood vessels respectively [28] or disorders in the autonomic nervous system [29]. The present experiments did not investigate these possible actions of IgG1. Recently, in human isolated airways, hyperresponsiveness has been observed after passive sensitization with an IgE-containing serum pool [30–32].

Highly purified IgG1-induced changes provides the opportunity to study the interaction between allergic factors and smooth muscles behaviour. In the present study, it is noteworthy that sensitization-induced modifications occurred prior to the specific antigen challenge. The present results in the guinea-pig provide some *in vitro* evidence that enhanced contractile responses under conditions of sensitization may be mediated by reagenic IgG1. It could be then postulated that hyperresponsiveness is a

general feature of the smooth muscles of the whole body. Further investigations of the cellular processes altered after sensitization will contribute to the understanding of the smooth muscle hyperresponsiveness with special attention to allergy and asthma.

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### References

- 1 Bidon J-C, Blin M, Gogny M, Vu AT, Jondet A. Enhancement of the responsiveness of vas deferens and ileum smooth muscle in sensitized guinea pigs. *In vitro* study. *Int Arch Allergy Immunol* 1994; 104:72–8.
- 2 Bidon J-C, Blin M, Gogny M, Vu AT, Jondet A. Strain-related differences in the tracheal responsiveness of sensitized guinea pig. *Int Arch Allergy Immunol* 1995; 106: 86–91.
- 3 Bidon J-C, Souilem O, Blin M *et al.* Effect of temperature reduction on the vas deferens hyperresponsiveness of sensitized mice. *J Auton Pharmacol* 1995; in press.
- 4 Morcillo EJ, Perpiña M, Esplugues J. Hyperresponsiveness to autacoids and autonomic drugs in lung parenchymal strips from sensitized guinea pigs. *Am Rev Respir Dis* 1984; 129:948–51.
- 5 Ortiz JL, Cortijo J, Sanz C *et al.* Cooling-induced contraction of trachea isolated from normal and sensitized guinea pigs. *Naunyn-Schmiedeberg's Arch Pharmacol* 1991; 343: 418–26.
- 6 Ortiz JL, Cortijo J, Sanz C *et al.* Non-specific hyper-reactivity to pharmacological stimuli in tracheal and lung parenchymal strips of actively sensitized guinea pigs. *J Pharm Pharmacol* 1989; 41:316–21.
- 7 Pascual R, Villanueva MM, Iriarte MCF *et al.* Role of endothelium in the responses to noradrenaline in normal and sensitized guinea-pig aorta. *J Auton Pharmacol* 1993; 13:105–13.
- 8 Regal JF. IgG vs IgE: Mediators of antigen-induced guinea pig tracheal contraction. *Immunopharmacology* 1984; 8: 111–9.
- 9 Regal JF. Immunoglobulin G- and immunoglobulin E-mediated airway smooth muscle contraction in the guinea pig. *J Pharmacol Exp Ther* 1984; 228:116–20.
- 10 Undem BJ, Buckner CK, Harley P, Graziano FM. Smooth muscle contraction and release of histamine and slow-reacting substance of anaphylaxis in pulmonary tissues isolated from guinea pigs passively sensitized with IgG1 or IgE antibodies. *Am Rev Respir Dis* 1985; 131:260–6.
- 11 Souhrada M, Souhrada JF. Specific reagenic antibody

- IgG1-induced changes of airway smooth muscle cells. *J Appl Physiol* 1988; 65:767-75.
- 12 Souhrada M, Souhrada JF. Immunologically-induced alterations of airway smooth muscle cell membrane. *Science* 1984; 225:723-5.
- 13 Souhrada M, Souhrada JF. The role of protein kinase-C in sensitization and antigen response of airway smooth muscle. *Am Rev Respir Dis* 1989; 140:1567-72.
- 14 Souhrada M, Souhrada JF. Effect of IgG1 and its fragments on resting membrane potential of isolated tracheal myocytes. *J Appl Physiol* 1993; 74:1948-53.
- 15 Martin LN. Separation of guinea pig subclasses by affinity chromatography on Protein A-Sepharose. *J Immunol Meth* 1982; 52:205-12.
- 16 Laëmlil UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T<sub>4</sub>. *Nature* 1970; 227:680-5.
- 17 Scheidegger JJ. Une microméthode de l'immunoélectrophorèse. *Int Arch Allergy* 1955; 7:103-10.
- 18 Tijssen P. Practice and theory of enzyme immunoassay. In: Tijssen P, eds. *Laboratory Techniques in Biochemistry and Molecular Biology*. Amsterdam: Elsevier, 1985:329-40.
- 19 Souilem O, Bidon J-C, Gogny M, Blin M, Jondet A. Effect of temperature reduction on the reactivity of the mouse vas deferens to adrenergic drugs. *Comp Biochem Physiol* 1992; 103C:557-61.
- 20 Chand N, Eyre P. The Schultz-Dale reaction: a review. *Agents Actions* 1978; 8:171-84.
- 21 De Lean A, Munson PJ, Rodbard D. Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. *Am J Physiol* 1978; 235:E97-E102.
- 22 Meddings JB, Scott RB, Fick GH. Analysis and comparison of sigmoidal curves: application to dose-response data. *Am J Physiol* 1989; 257:G982-G989.
- 23 Bennacerraf B, Ovany Z, Bloch KJ, Franklin EC. Properties of guinea pig 7S antibodies. I Electrophoresis separation of two types of guinea pig 7S. *J Exp Med* 1963; 117:937-49.
- 24 Mansour S, Daniel EE. Responsiveness of isolated tracheal smooth muscle from normal and sensitized guinea pigs. *Can J Physiol Pharmacol* 1987; 65:1942-50.
- 25 Featherstone RL, Hutson PA, Holgate ST, Church MK. Active sensitization of guinea pig airways in vivo enhances in vivo and in vitro responsiveness. *Eur Respir J* 1988; 1:839-45.
- 26 Perpiña M, Cortijo J, Fornas E, Palau M, Ortiz JL, Morcillo E. Hyperreactivity and Ca movements in sensitized guinea pig tracheal muscle. *Eur Respir J* 1991; 4:450-7.
- 27 Vu AT, Bidon J-C, Jondet A. Histological and histochemical localization of the mast cells in four organs of guinea pig: aorta, trachea, taenia coli and vas deferens. *Biol Cell* 1992; 75:6a-7a.
- 28 Iriarte CF, Pascual R, Villanueva MM et al. Epithelium modulates the reactivity of sensitized guinea-pig trachea: influence of the surface of drug entry. *J Pharm Pharmacol* 1991; 43:392-5.
- 29 Barnes PJ. Neural mechanisms in asthma. *Br Med Bull* 1992; 48:149-68.
- 30 Black JL, Marthan R, Armour CL, Johnson PRA. Sensitization alters contractile responses and calcium influx in human airway smooth muscle. *J Allergy Clin Immunol* 1989; 84:440-7.
- 31 Marthan R, Crevel H, Guénard H, Savineau J-P. Responsiveness to histamine in human sensitized airway smooth muscle. *Respir Physiol* 1992; 90:239-50.
- 32 Ben-Jebria A, Marthan R, Rossetti M, Savineau JP. Effect of passive sensitization on the mechanical activity of human isolated bronchial smooth muscle induced by substance P, neurokinin A and VIP. *Br J Pharmacol* 1993; 109:131-6.

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