

Antigen-specific tachycardia and hypotension in rodents

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Summary

Tachycardia and hypotension, two cardiovascular responses to anaphylaxis, were specifically induced by antigen in mice and rats, respectively. Intravenous injection of poly (Glu⁶⁰Ala³⁰Tyr¹⁰) (GAT) elicited tachycardia within 30–40 sec in GAT-primed B6 mice. Moreover, a minute amount of GAT (0.2 µg) was enough to sensitize the mice to subsequent GAT-induced tachycardia. Challenging doses ranging from 100 ng to 500 µg could elicit tachycardia. The kinetics of tachycardia induction was different from that of antibody production or delayed-type hypersensitivity. Tachycardia was induced from day 6 after immunization, while delayed-type hypersensitivity developed as early as day 4, and anti-GAT antibodies were undetectable on day 6 and would not reach a maximum until day 8. Specific antigen-induced hypotension was also observed in rats. Furthermore, cardiovascular changes in both species could be passively transferred by heat-treated (56°C, 30 min) sera from immunized animals. These benchmarks of antigen-induced cardiovascular changes in mice or rats could be used as models to study the immune control of cardiovascular changes in anaphylactic responses.

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Introduction

Antigen-induced immune responses are evaluated by various parameters such as antibody production, delayed-type hypersensitivity, cytotoxic T-cell activity, T-cell proliferation, cytokine production, etc. The heart is recognized as a target organ in systemic allergic reaction [1,2]. In the guinea pig, cardiac anaphylaxis is associated with sinus tachycardia, atrioventricular conduction block and increased ventricular automaticity [3]. However, it has been attributed to immediate-type (or IgE-mediated) hypersensitivity, and is believed to involve the release of histamine from mast cells. In addition, sulphidopeptide leukotrienes and platelet-activating factor have been demonstrated to be generated on anaphylactic reaction of heart tissue in the guinea pig [4–6]. A number of studies in rats, guinea pigs, and rabbits, both *in vivo* and *in vitro*, have attempted to define the association between cardiac responses and anaphylaxis [7–9]. In humans, several cardiac dysfunctions often occur during hypersensitivity

reactions [10–13]. Inbred strains of mice are advantageous in the study of the genetic control of immune responses, and might generate valuable information for the study of the genetic regulation of anaphylaxis. Recently, a novel type of antigen-specific hypersensitivity has been elicited within 1 hr of antigen challenge [14–16], and was referred to as early-type hypersensitivity (ETH). This ETH can be transferred by either immune sera [16] or T cell-derived small molecular weight factor [14], and was therefore thought to be different from IgE-mediated hypersensitivity or delayed-type hypersensitivity (DTH). The ETH was induced by most antigens tested and is also under the Ir gene control. This study showed that the time interval required for various types of hypersensitivity to develop after immunization varied. DTH required 4–6 days, ETH, 8–11 days and immune complex-type hypersensitivity 18–21 days. Although ETH required 3 days more to develop than DTH, it lasted longer once developed, and the pattern of the response was inversely related to DTH. The ETH response involved an increased vasopermeability and oedema after subcutaneous injection in the ear. However, if antigen is given intravenously, the mouse developed anaphylactic symptoms such as

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lethargy, tachypnoea and myoclonic jerk. The present study reports on antigen-induced cardiovascular changes of anaphylaxis in the mouse and rat.

Materials and methods

Animals

Breeders of BALB/c (H-2^d), and B6 (H-2^b) strains were purchased from The Jackson Laboratory, Bar Harbor, Maine, USA. Breeders of Wistar rats were derived from The Imamichi Institute for Animal Reproduction, Ibarake, Japan. They were maintained on standard laboratory chow and water *ad libitum* in the animal facility of the Medical College, Cheng Kung University. The animals were raised and cared for following the guidelines set up by the National Science Council of ROC. The mice (ranging from 8 to 12 weeks) and rats (body weight around 200 g) were used in the experiments.

Reagents

Poly (Glu⁶⁰Ala³⁰Tyr¹⁰) (GAT), horse γ -globulin (HoGG) and urethane were purchased from Sigma Chemical Co., St Louis, Missouri, U.S.A. Sodium pentobarbital (Nembutal) was purchased from Abbott Laboratories (North Chicago, Illinois, U.S.A.).

Electrocardiogram and blood pressure measurement in anaphylaxis

Mice were injected subcutaneously at the base of the tail with 50 μ l of an emulsion containing 20 μ g GAT in complete Freund's adjuvant (CFA) (Difco Laboratories, Detroit, Michigan, U.S.A.) [14]. On day 20, anaphylactic responses were induced by injecting 500 μ g of GAT diluted in 100 μ l of saline intravenously. In some experiments, either the doses of immunization and challenge or day were varied. To monitor cardiac changes in anaphylaxis, mice were anaesthetized with pentobarbital at 60 mg/kg body weight by intraperitoneal injection. The electrocardiogram (ECG) of standard limb leads or heart rates were monitored through Gould ECG/Biotach Amplifiers (Model 13-4615-65) and recorded on a two-channel recorder (Gould 2200S portable ink recorder, Gould Inc., Cleveland, Ohio, U.S.A.). Rats were used for the measurement of blood pressure because of the technical difficulty of blood pressure measurements in mice. The rats were immunized subcutaneously at the base of the tail with 100 μ g of GAT in CFA. On day 20, anaphylactic responses were induced by injecting 2 mg of GAT diluted in 100 μ l of saline intravenously. To monitor blood pressure changes in anaphylaxis, the rats were anaesthetized with urethane at 5 g/kg body weight by

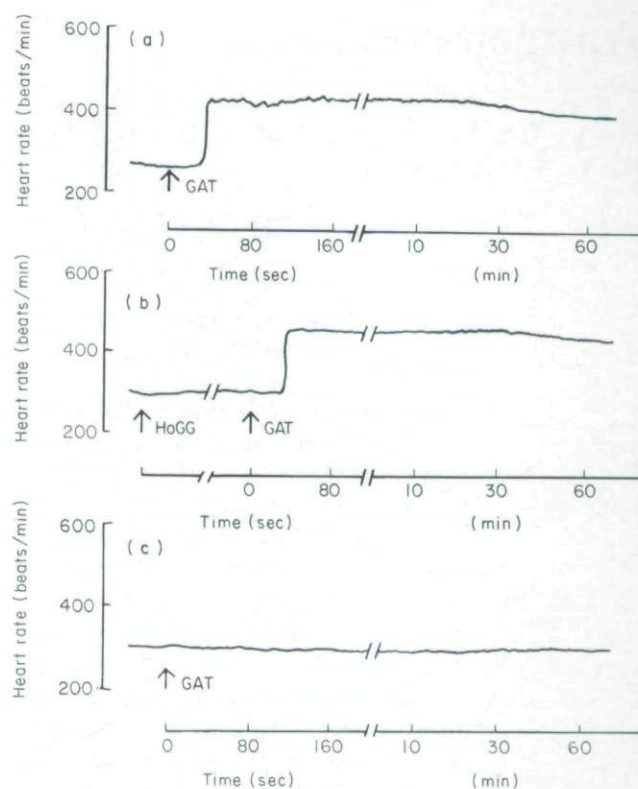


Fig. 1. Specific antigen-induced tachycardia in B6 mice. B6 mice were immunized subcutaneously with 20 μ g of GAT/CFA at the base of the tail. Twenty days later, mice were injected intravenously with 500 μ g of GAT or HoGG and heart rate was monitored with a polygraph. (a) GAT challenge in GAT-primed mouse. (b) HoGG challenge followed by GAT challenge 10 min later in GAT-primed mouse. (c) GAT challenge in naive mouse.

intraperitoneal injection. Arterial blood pressure was monitored via a catheter (Intramedic PE 50) which was cannulated into the femoral artery and connected to a pressure transducer (Gould) and a two-channel recorder (Gould).

Delayed-type hypersensitivity

Groups of five mice were injected subcutaneously at the base of tail with 50 μ l of 20 μ g GAT in CFA [14]. On various days after immunization, hypersensitivity responses were elicited by injecting 10 μ g of GAT in 10 μ l of saline into the dorsal surface of the ear using a 100 μ l Hamilton syringe fitted with a 27-gauge needle. Twenty-four hours after the ear challenge the increment in ear thickness over the prechallenge state was determined with a Federal model P11 engineer's micrometer (Providence, Rhode Island, U.S.A.) and the results were expressed in units of 10^{-3} mm.

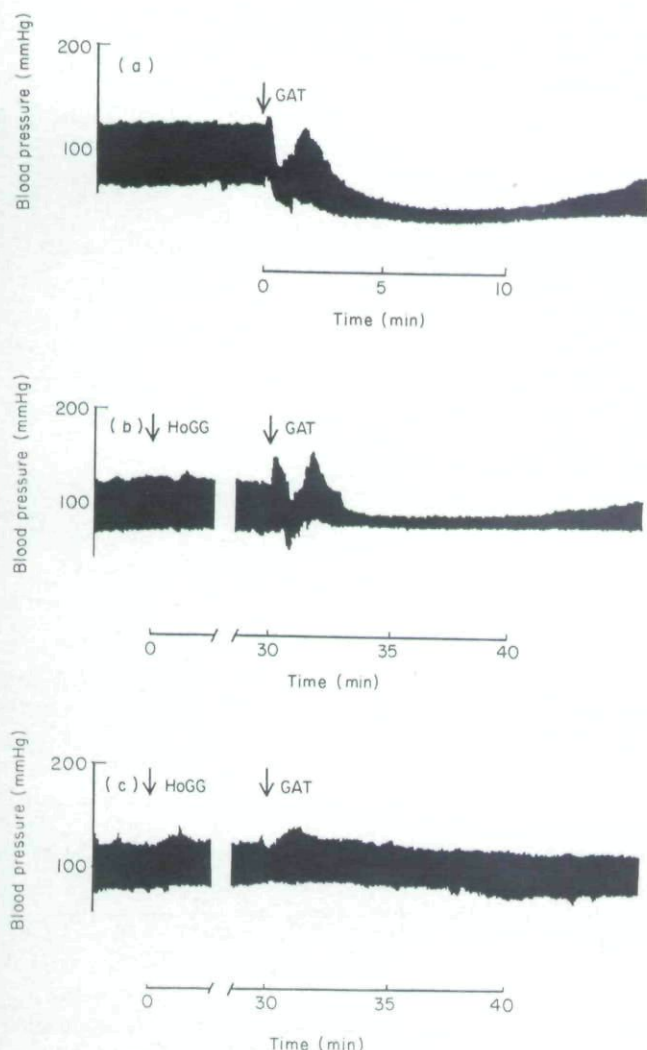


Fig. 2. Specific antigen-induced hypotension in Wistar rats. Wistar rats were immunized subcutaneously with 100 μ g of GAT/CFA. Twenty days later, the rats were injected intravenously with 2 mg of GAT or HoGG and blood pressure was monitored with a polygraph. (a) GAT challenge in GAT-primed rat. (b) HoGG challenge followed by GAT challenge in GAT-primed rat. (c) HoGG challenge followed by GAT challenge in naive rat.

Production of anti-GAT antibodies

Groups of five mice were injected subcutaneously at the base of tail with 50 μ l of 20 μ g GAT in CFA. Mice were bled from the retro-orbital plexus on various days after immunization. Anti-GAT antibody titre was measured with a solid-phase sandwich radioimmunoassay as described previously [15]. Briefly, polyvinyl chloride plates (Costar, Cambridge, Massachusetts, U.S.A.) were coated overnight with 50 μ l GAT (100 μ g/ml in 0.01 M bicarbonate buffer, pH 8.6). Plates were then blocked with 1%

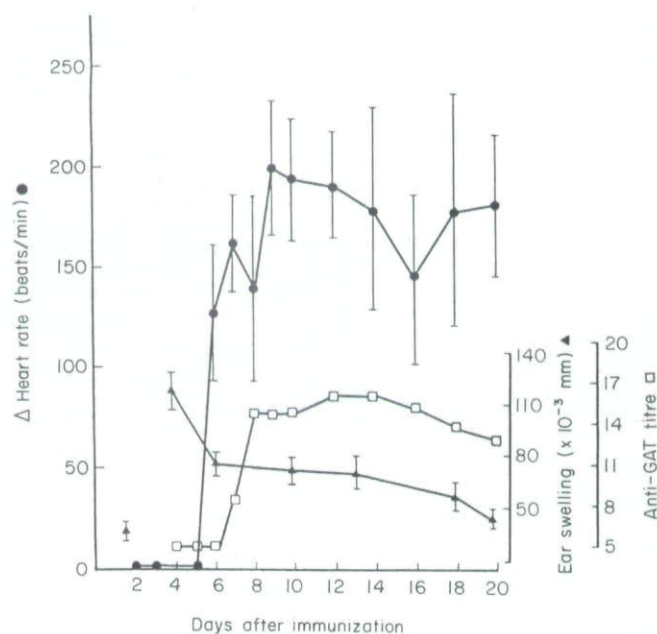


Fig. 3. Kinetics of tachycardia induction, antibody production and delayed-type hypersensitivity in B6 mice. Groups of B6 mice were immunized subcutaneously with 20 μ g of GAT/CFA at the base of the tail. On various days after immunization, mice were injected intravenously with 500 μ g of GAT and heart rate was monitored with a polygraph and expressed as change in heart rate after challenge. In separate experiments, sera were collected from immunized mice and the anti-GAT antibody titre was determined and expressed as the reciprocal of the $\log_2$ of the highest serum dilution to yield $2.5 \times$ the counts of preimmunization serum. Delayed-type hypersensitivity response was determined as ear swelling 24 hr after antigen challenge. Bars indicated the standard deviation ($n=5$).

haemoglobin in phosphate buffered saline (PBS) for 1 hr. Fifty microlitres of twofold serially diluted sera were added to wells in triplicate, and incubated for 4 hr at room temperature. Wells were then rinsed with PBS, after which 50 μ l of [^{125} I]-rabbit anti-mouse IgG (2×10^5 c.p.m.) were added, and incubated for 4 hr at room temperature. The wells were rinsed with PBS three times, and individual cells were cut with electric wire and counted in an automatic gamma counter (LKB-Walilac 1282 Compu-gamma). The highest serum dilution yielding $2.5 \times$ the counts of preimmunization serum was considered the titre and was expressed as the reciprocal of $\log_2$ of the dilution.

Passive transfer of anaphylaxis

Naive mice or rats were injected intravenously with 0.2 ml or 3 ml, respectively, of anti-GAT immune sera. Half an hour later, they were injected intravenously with 500 μ g (mice) or 2 mg (rats) of either GAT or HoGG. Changes in either heart rate or blood pressure were then monitored.

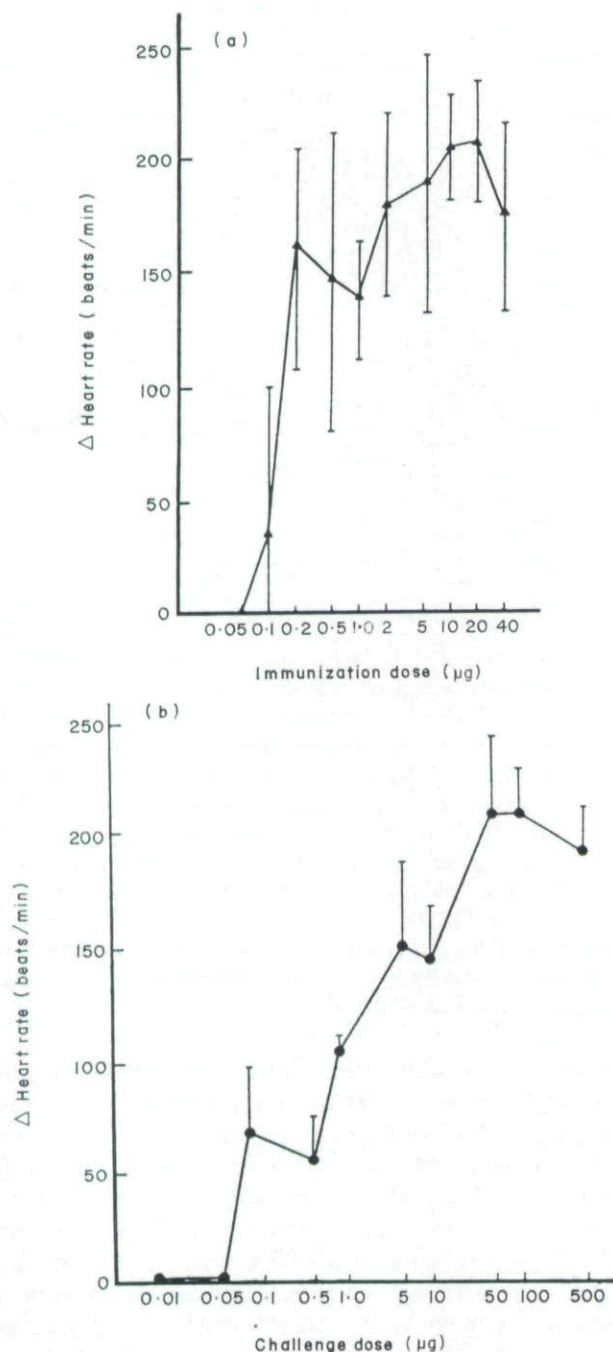


Fig. 4. Immunization and challenge doses to develop tachycardia in B6 mice. (a) Groups of B6 mice were immunized subcutaneously with different doses of GAT/CFA at the base of the tail. Twenty days later, they were injected with 500 μ g of GAT intravenously and heart rate was monitored with a polygraph and expressed as change in heart rate. (b) Groups of B6 mice were immunized subcutaneously with 20 μ g of GAT/CFA at the base of the tail. Twenty days later, mice were injected intravenously with various doses of GAT and heart rate was monitored with a polygraph and expressed as change in heart rate. Bars indicated the standard deviation ($n=5$).

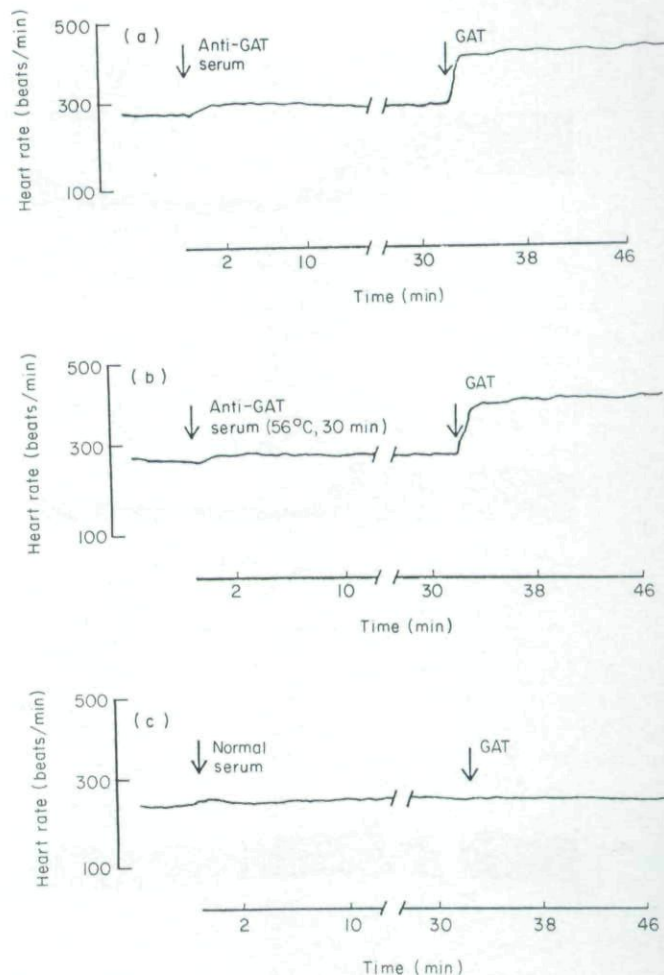


Fig. 5. Passive transfer of tachycardia in B6 mice. Naive B6 mice were injected intravenously with 0.2 ml of (a) anti-GAT sera derived from GAT-boosted BALB/c mice, (b) identical sera that had been heated at 56°C for 30 min, or (c) normal sera. Half an hour later, mice were injected intravenously with 500 μ g of GAT and heart rate was monitored with a polygraph.

Results

Antigen-induced cardiovascular changes in anaphylaxis were monitored in rodents. Recordings from typical reactions are shown in Figs 1 (mice) and 2 (rats). Intravenous injection of GAT in GAT-primed B6 mice caused increased heart rate in 30–40 seconds (Fig. 1a), and the tachycardia persisted for at least 1 hr. This induction was apparently antigen-specific, since an irrelevant antigen, HoGG failed to induce tachycardia in GAT-primed mice (Fig. 1b), and GAT induced no tachycardia in naive mice (Fig. 1c). Blood pressure changes in anaphylaxis were monitored in the larger rat model. Intravenous injection of GAT caused a decrease in blood pressure in GAT-primed Wistar rats (Fig. 2a). This

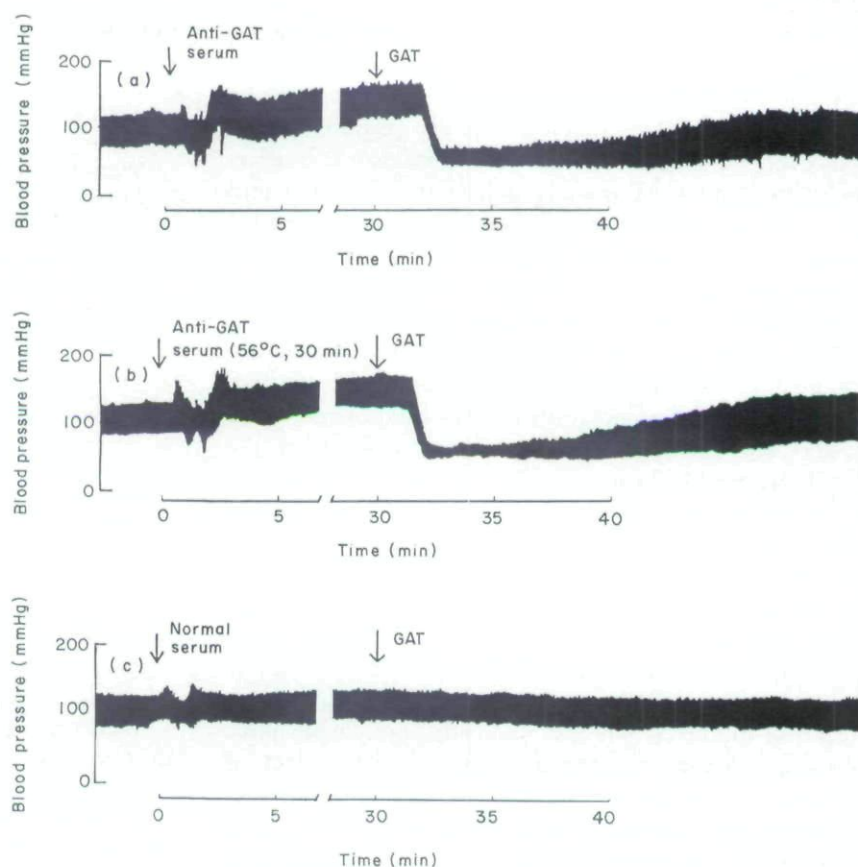


Fig. 6. Passive transfer of hypotension in Wistar rats. Naive Wistar rats were injected intravenously with 3 ml of (a) anti-GAT sera derived from GAT-primed Wistar rat, (b) identical sera that had been heated at 56°C for 30 min, or (c) normal sera. Half an hour later, rats were injected intravenously with 2 mg of GAT and the blood pressure was monitored with a polygraph.

induction of hypotension was also antigen specific, as shown in Figs 2b and 2c.

The kinetics of tachycardia induction, DTH and antibody production in mice were also investigated. Groups of five B6 mice were immunized with GAT in CFA, and these parameters were evaluated on various days after immunization. As shown in Fig. 3, the tachycardia developed on day 6 and lasted for as long as 90 days (data not shown). However, DTH appeared as early as day 4, declining by day 20, whereas anti-GAT antibodies were undetectable on day 6 and did not reach a maximum until day 8. The immunization and challenge doses for tachycardia were also determined. As low as 0.2 µg of GAT could sensitize B6 mice to develop tachycardia in response to subsequent GAT challenge (Fig. 4a), while the challenging dose appeared to range from 100 ng to 500 µg of GAT (Fig. 4b).

To determine whether immune sera could transfer the anaphylactic tachycardia and hypotension, naive rodents were given anti-GAT antisera one half hour before being challenged with GAT intravenously. Recordings from typical reactions are shown in Figs 5 and 6. Tachycardia was induced by GAT in serum-treated mice in a fashion very similar to that induced in actively immunized mice

(Fig. 5a). Heat treatment (56°C, 30 min) did not destroy the transfer activity of these antisera (Fig. 5b). Hypotension in rats could also be passively transferred by antisera, and this activity was also heat resistant (Fig. 6). The passive transfer of cardiovascular changes by heat-resistant sera suggests that non-IgE factor(s) may play a role in mediating the cardiovascular changes of anaphylaxis.

Discussion

We have previously reported an antigen-specific hypersensitivity which does not fit into the traditional classification of hypersensitivity [14]. Subcutaneous injection of antigen in the mouse ear caused increased vasopermeability in 15 min with oedema [16]. This hypersensitivity was called early-type hypersensitivity. This ETH could be transferred by heat-treated immune sera [16] or small molecular weight T-cell derived factors [14]. The induction of ETH is under the control of the Ir gene. The present study has shown that when a synthetic polymer, GAT, was given intravenously into GAT-primed mice or rats, anaphylactic responses such as tachycardia and hypotension developed in an antigen-specific manner.

Furthermore, passive transfer of heat-resistant sera could mediate these cardiovascular changes, as well as ETH.

It has been known for many years that the heart can be the target organ in systemic allergic reactions [1,2]. However, experimental cardiac anaphylaxis was primarily investigated using animals such as the guinea pig, and IgE and histamine were the principle mediators in this antigen-induced response [17]. In the rabbit, platelet activating factor was responsible for the IgE-mediated anaphylaxis [7,18]. Munoz and Bergman reported that mice undergoing anaphylactic shock died of circulatory failure due to loss of effective blood volume. This effective loss of volume was not only due to dilation of blood vessels but also to an increased permeability of the capillaries which allowed fluids to escape [19]. Martin *et al.* reported cardiopulmonary alterations (elevation of heart rate, reduction of pulmonary dynamic compliance and conductance) in mast cell-deficient W/W^v, but not in congenic normal (+/+) mice after treating them with goat anti-IgE antibody [20]. However, Jacoby *et al.* showed that systemic anaphylaxis could be readily induced in the W/W^v mouse, and that the anaphylactic response in this strain was clinically indistinguishable from that of the +/+ mouse, despite a relative lack of mast cells in the former [21]. Passive cutaneous anaphylaxis was ascribed to either IgE or IgG1 antibody [22,23]. In our study, the expression of antigen-specific ETH, the different onset times of tachycardia, DTH and antibody production, and the passive transfer by heat-resistant sera all seem to suggest that histamine and IgE may not be major mediators of antigen-induced systemic anaphylaxis in mice. The hypotension response in rats might relate to the dilatation of blood vessels and the increased vasopermeability, which are characteristics of ETH. The observation that the induction of tachycardia is rapid and intense and can develop as early as day 6 after immunization is intriguing. Possible mechanisms include direct complement activation, thrombotic events and endothelial cell-induced responses which are under current investigation. Nevertheless, these responses might have physiological relevance to clinical non-IgE-mediated anaphylaxis [24–27], and the measurement of tachycardia or hypotension in mice or rats provides a model to study the mediators involved in antigen-induced cardiovascular changes and the genetic control of anaphylaxis.

Acknowledgments

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