Motor activity as an unbiased variable to assess anaphylaxis in allergic rats

Mar Abril-Gil¹, Alba Garcia-Just¹, Trinitat Cambras¹, Francisco J Pérez-Cano^{1,2}, Cristina Castellote^{1,2}, Àngels Franch^{1,2} and Margarida Castell^{1,2}

¹Departament de Fisiologia, Facultat de Farmàcia, Universitat de Barcelona, Av. Joan XXIII s/n, E-08028 Barcelona, Spain; ²Institut de Recerca en Nutrició i Seguretat Alimentària (INSA-UB), E-08028 Barcelona, Spain
Corresponding author: Margarida Castell. Email: margaridacastell@ub.edu

Abstract

The release of mediators by mast cells triggers allergic symptoms involving various physiological systems and, in the most severe cases, the development of anaphylactic shock compromising mainly the nervous and cardiovascular systems. We aimed to establish variables to objectively study the anaphylactic response (AR) after an oral challenge in an allergy model. Brown Norway rats were immunized by intraperitoneal injection of ovalbumin with alum and toxin from *Bordetella pertussis*. Specific immunoglobulin (Ig) E antibodies were developed in immunized animals. Forty days after immunization, the rats were orally challenged with the allergen, and motor activity, body temperature and serum mast cell protease concentration were determined. The anaphylaxis induced a reduction in body temperature and a decrease in the number of animal movements, which was inversely correlated with serum mast cell protease release. In summary, motor activity is a reliable tool for assessing AR and also an unbiased method for screening new anti-allergic drugs.

Keywords: Allergy, anaphylaxis, mast cell, motor activity, rat

Experimental Biology and Medicine 2015; 240: 1373–1377. DOI: 10.1177/1535370215573393

Introduction

IgE against allergens is the antibody responsible for most allergies. IgE coats the surface of mast cells and the subsequent exposure to the same allergen triggers the release of the mast cell intragranular content – mainly histamine, proteases and other enzymes – and later mediators such as cytokines and leukotrienes, which lead to the allergic manifestations. As well known, the release of mediators produces physiological changes such as vasodilatation, an increase in vascular permeability, and smooth muscle contraction, which affects the skin, respiratory and gastrointestinal systems or even the nervous and cardiovascular systems.^{1,2} The most severe form of allergic reaction is anaphylaxis, which affects at least two organic systems, sometimes resulting in an anaphylactic shock, which is characterized by cardiovascular and nervous disorders including hypotension, an increase in heart rate and hypothermia, which can result in death.^{3,4}

Animal models are extremely valuable for the assessment of allergenic potential and for the screening of anti-allergic drugs. In this context, Brown Norway (BN) rats have been described as a high-IgE responder strain and, to a degree, resemble atopic humans in their predisposition

to react more readily to antigens with an overproduction of IgE.^{3,5,6} Anaphylaxis can be studied in rodents by measuring ear-swelling response, body temperature, and respiration patterns and through blood pressure monitoring, as well as changes in the animals' behaviour using scoring systems or, in the most severe cases, the number of deaths. Likewise, different mediators such as histamine or mast cell protease release, or serum-specific IgG and IgE have been determined.^{5,7} In this study, we aimed to establish an unbiased variable that would allow us to objectively assess the anaphylactic response (AR) after an oral challenge with ovalbumin (OVA) in BN rats, focusing on changes in animal behaviour.

Materials and methods

Rat allergy induction and ethical approval

Three-week-old female Brown Norway (BN) rats (Janvier, Le Genest-Saint-Isle, France) were maintained under conditions of controlled temperature and humidity in a 12:12 light:dark cycle. After a one-week acclimation period, the rats were randomized into two groups: allergy and reference ($n=8$ in each one). The allergy was induced using an emulsion of ovalbumin (OVA, grade V, Madrid,

Sigma-Aldrich, Spain) as allergen, in alum (Imject®; Pierce, Rockford, IL, USA) as adjuvant and toxin from *Bordetella pertussis* (tBp; Sigma-Aldrich) to promote IgE synthesis.⁸ Each rat received only one intraperitoneal (i.p.) injection with 0.5 mL of the emulsion containing 50 µg of OVA, 50 ng of tBp, and 2.5 mg of Imject® (Figure 1a). Before this injection and five weeks later, blood samples were collected from the saphenous vein to determine specific IgE concentration. Forty days from the beginning of the study, anaphylaxis was induced. Two days later, rats were sacrificed and blood samples were obtained by heart puncture. Experimental procedures were reviewed and approved by the Ethical Committee for Animal Experimentation of the University of Barcelona (ref. 359/12).

Induction of anaphylaxis

The day before anaphylaxis induction, both groups were deprived of food overnight. The rats received 2 mL of OVA (100 mg/mL) orally to induce an AR. Motor activity was immediately assessed for 21 min. Rectal temperature was determined (digital thermometer, OMRON Healthcare Hoofddorp, the Netherlands). Blood was collected before oral challenge and every 30 min up to 2 h post-AR induction from the saphenous vein to determine serum rat mast cell protease II (RMCP-II) concentration (Figure 1(b)).

Measurement of motor activity

Motor activity was measured by using individual cages in an isolated room, with an activity meter that included two perpendicular infrared beams, which crossed the cage 6 cm

above the floor as has been reported previously⁹ (Figure 1(b)). Two motor activity measures were performed: the first (basal) 24 h before and the second immediately after the oral challenge. Activity counts were recorded using time frames of 1 min for 21 min. To stimulate rat movements, 8 min after the beginning of the measurement the lights were turned off for 5 min and then turned on until the end of the measurement. The results refer to the movements in three time phases: pre-darkness, darkness, and post-darkness, as well as the entire period. The percentage of motor activity decreases after AR induction was calculated with respect to the basal measurement in each studied phase and the whole period.

Quantification of anti-OVA IgE antibodies

OVA-specific IgE concentrations were quantified in serum samples collected before allergy induction, and five and six weeks later by ELISA as previously described.¹⁰

Quantification of rat mast cell protease

Serum RMCP-II concentration was measured using a commercial ELISA set (Moredun Animal Health, Edinburgh, UK) with slight modifications. In brief, ELISA plates were coated with anti-RMCP-II antibody (overnight, 4°C). After blocking and washing, appropriately diluted serum samples were incubated for 3 h. After washing, peroxidase-conjugated anti-RMCP-II antibody was incubated for 2 h. Finally, a 3,3',5,5'-tetramethylbenzidine solution (with H₂O₂) was added and the optical density (OD) was

Figure 1 Experimental protocol. (a) Time-course of the experimental design including the points of sample collection. (b) Motor activity assessment 24 h before (day 39) and immediately after the induction of anaphylaxis (day 40) with the determinations carried out. Two kinds of infrared beams are represented: E is the emitter and R is the receiver

measured (microtiter plate photometer, Labsystems Multiskan, Helsinki, Finland).

Statistical analysis

The software package IBM SPSS Statistics 20 (SPSS Inc., Chicago, IL, USA) was used. The Levene's and the Kolmogorov-Smirnov tests were applied to assess variance equality and normal distribution, respectively. One- and two-way ANOVA tests were used to study the effect of group and group $\times$ time interaction, respectively. The motor activity data were analysed by two-way ANOVA for repeated measures considering the group (allergy group *vs.* reference group) and time as the interacting factors followed by Bonferroni's *post hoc* test. To evaluate the correlation among studied variables, Pearson's coefficient (ρ) was applied. To analyse the results from anti-OVA IgE concentration, a non-parametric test (Mann-Whitney U) was used due to non-variance homogeneity. RMCP-II and body temperature results were analysed by one-way ANOVA. Differences were considered statistically significant for $p < 0.05$.

Results

Serum anti-OVA IgE antibodies

Before immunization, both reference and allergy groups had no detectable OVA-specific IgE antibodies (Figure 2). Five weeks after immunization (before AR induction), the allergy group had remarkable anti-OVA IgE titres ($P < 0.001$), and at day 42 these values remained high.

Serum mast cell-released mediators

After AR induction, the RMCP-II concentration in reference animals was about 1266.51 ± 19.90 ng/mL (mean $\pm$ S.E.M.). The allergy group showed about a threefold increase in RMCP-II concentration (4052.69 ± 20.48 ng/mL) compared to that in reference animals ($P < 0.0001$). This increase was already observed at 30 min post-challenge and remained for at least 2 h (Figure 3(a)). There was a direct correlation between the amount of RMCP-II 30 min after AS and the

concentration of specific IgE antibodies at days 35 ($\rho = 0.873$, $P < 0.01$) and 42 ($\rho = 0.716$, $P < 0.05$).

Body temperature

The body temperature in the allergy group was about 2° lower than that of the reference group ($P < 0.05$) over 2 h after AR induction (Figure 3(b)).

Motor activity

One day before AR induction, motor activity data showed that animals from the allergy group had fewer movements than those in the reference group throughout the whole studied period and taking into account the three established phases (pre-darkness, darkness and post-darkness, $P < 0.01$, Figure 4a). Both groups presented the maximal activity in the first minutes, and the longer the measurement time, the quieter the animals were ($P < 0.0001$ for time). There was no significant effect in the interaction group $\times$ time.

After AR induction, both groups exhibited lower motor activity (Figure 4(b)) than that observed in the previous

Figure 2 Serum OVA-specific IgE antibodies in allergy (black bars) and reference (white bars) groups during post-immunization period. Results are expressed as mean $\pm$ SEM ($n = 8$). * $P < 0.001$ between both groups

Figure 3 (a) Serum RMCP-II relative concentration after AS induction in allergy (black bars) and reference (white bars) groups. Each bar represents the mean $\pm$ SEM ($n = 8$). (b) Body temperature after AS induction in allergy (●) and reference (○) groups. Results are expressed as mean $\pm$ SEM ($n = 8$). * $P < 0.05$, ** $P < 0.0001$ between both groups

Figure 4 Motor activity results during the 21-min period: (a) 24 h before the AS induction; (b) immediately after AS induction; and (c) percentage of motor activity decrease after AS induction referring to pre-darkness, darkness, post-darkness, and the whole period. Allergy group (● or black bars) and reference group (○ or white bars). Results are expressed as mean $\pm$ SEM ($n = 8$). * $P < 0.05$ between both groups. In (a) and (b), shadow period corresponds to darkness

measurement (Figure 4(a)). For the whole studied period and for each established phase, the allergy group showed a decrease in the number of movements compared to the reference group ($P < 0.0001$ for groups). In the pre-darkness phase but not later, a relevant interaction group $\times$ time was observed ($P < 0.05$). There was an inverse correlation between the number of movements and the amount of RMCP-II released 30 min after oral challenge ($\rho = -0.788$, $P < 0.01$).

With regard to the percentage of motor activity decrease after AR induction (Figure 4(c)), reference animals presented a reduction of about 40–60% whereas this reduction was significantly higher in the animals with anaphylaxis, which achieved a decrease of around 80% ($P < 0.05$ for the entire period and the pre-darkness phase).

Discussion

In the current study, an anaphylaxis was induced in allergic rats and this was objectively assessed by changes in rat behaviour, such as motor activity, together with a decrease in body temperature and the appearance of mast cell mediators in serum. In Brown Norway (BN) rats, the immunization with ovalbumin (OVA) plus adjuvant together with toxin from *Bordetella pertussis* allows an allergy rat model

to be obtained that is characterized by high and permanent serum anti-OVA IgE production as reported previously.¹⁰ After 5–6 weeks of immunization, oral administration of high amounts of OVA could challenge an anaphylaxis that caused changes in several physiological systems.

The anaphylaxis is a systemic response of the immune system due to a general mast cell release of mediators and affects multiple target organs, including the cardiovascular and nervous systems. Systemic anaphylaxis can be monitored by quantifying mast cell mediators in serum. A good mast cell mediator in the current study, in agreement with others,^{11,12} was mast cell protease II (RMCP-II). This enzyme could be quantified in healthy animals and it increased threefold in allergic animals, remaining for at least 2 h after challenge. The release of mast cell mediators produces vasodilatation and increases vascular permeability,⁴ which lowers body temperature.^{1,3} In the current study, mast cell degranulation in orally challenged animals could also be assessed by means of a drop in body temperature that lasted for at least 2 h after challenge. These results agree with others showing the correlation between changes in body temperature and anaphylaxis symptom scores.¹²

On the other hand, it has long been established that allergy is associated with behavioural changes. Mast cells found in

the brain are able to regulate many physiological functions within the central nervous system.¹³ In this sense, it has been described that the allergen challenge in rodents with a respiratory allergy induces an anxiety-like behaviour and a reduction in social interactions.¹⁴ As a part of the evaluation of anaphylaxis in rodents, some variables concerning animal behaviour have been reported. Such variables include scratching or rubbing around the nose or head, and wheezing or abdominal cramping, which are usually evaluated by means of scoring systems with other manual or subjective indexes.¹⁵ These methods require the observation of each animal and this can be subject to personal interpretation. Here, a new system is introduced to assess an AR in rodents that consists in recording objectively the number of movements by means of an activity meter. This method allows behaviour changes related to the reduction in activity in anaphylaxis-induced animals to be quantified in an objective way. It also has the advantage of having monitored a higher number of isolated animals at the same time.

In the current study, we aimed to quantify movements after AR induction and, in order to establish basal data, animal movements were recorded 24 h before the allergen challenge. Surprisingly, we could notice that the immunized animals showed fewer movements than the reference rats even before the anaphylaxis induction. The reason of such decrease cannot be explained from the data in the current study, but it could be related to the relationship between allergy and behaviour.^{13,16,17} After allergen challenge, there was a decrease in the number of movements of both reference and allergic rats compared to those produced the day before in the same conditions. This general decrease could be attributed to the blood sample collection carried out minutes before oral challenge in every animal. However, this decrease was significantly higher in allergic rats than in non-immunized animals, showing therefore the anaphylaxis response, and was more patent in the first few minutes after challenge, when animals were placed in a new cage and then explored the new environment. The movements recorded after the anaphylaxis induction inversely correlate with the release of RMCP-II from mast cells measured in samples obtained few minutes later, suggesting that the higher the mast-cell degranulation, the quieter the animals were.

In conclusion, this study offers a new objective variable, motor activity, for assessing the AR in an allergy model induced in rodents. This tool represents a reliable and unbiased way to screen new anti-allergic drugs.

Author contributions: FPC, CC, AF, and MC were responsible for designing the study, and MAG, AGJ, AF, and MC carried out the experiments. TC was responsible for the activity motor assessment. All authors approved the final version of the manuscript.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Antoni Díez-Noguera for lending us the activity meters and Malen Massot-Cladera for

her technical assistance. This study was supported by grants from the Spanish Ministries of Science and Innovation (AGL2008-02790) and Economy and Competitivity (AGL2011-24279).

REFERENCES

1. Makabe-Kobayashi Y, Hori Y, Adachi T, Ishigaki-Suzuki S, Kikuchi Y, Kagaya Y, Shirato K, Nagy A, Ujiike A, Takai T, Watanabe T, Ohtsu H. The control effect of histamine on body temperature and respiratory function in IgE-dependent systemic anaphylaxis. *J Allergy Clin Immunol* 2002;**110**:298–303
2. Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. *Nature* 2008;**454**:445–54
3. Bellou A, Saint-Laudy J, Knippels L, Montémont C, Vauthier E, Gerard P, Pellegrom H, Koerkamp EK, Lesesve JF, Guéant JL, Lambert H, Mallié JP. Brown Norway rat ovalbumin-specific immunoglobulin E antibodies increase the human basophil expression of CD63 marker. *Scand J Immunol* 2003;**57**:271–8
4. Simons FE. Anaphylaxis. *J Allergy Clin Immunol* 2010;**125**:S161–81
5. Jia XD, Li N, Wu YN, Yang XG. Studies on BN rats model to determine the potential allergenicity of proteins from genetically modified foods. *World J Gastroenterol* 2005;**11**:5381–4
6. Li Z, Gao Y, Wang H, Liu Z. A rat model of Shuang Huang Lian injection-induced anaphylaxis. *Asian Pac J Allergy Immunol* 2010;**28**:185–91
7. Akiyama H, Sato Y, Watanabe T, Nagaoka MH, Yoshioka Y, Shoji T, Kanda T, Yamada K, Totsuka M, Teshima R, Sawada J, Goda Y, Maitani T. Dietary unripe apple polyphenol inhibits the development of food allergies in murine models. *FEBS Lett* 2005;**579**:4485–91
8. Dong W, Selgrade MK, Gilmour MI. Systemic administration of Bordetella pertussis enhances pulmonary sensitization to house dust mite in juvenile rats. *Toxicol Sci* 2003;**72**:113–21
9. Albert N, da Silva C, Díez-Noguera A, Cambras T. Different adaptation of the motor activity rhythm to chronic phase shifts between adolescent and adult rats. *Behav Brain Res* 2013;**252**:347–55
10. Abril-Gil M, Massot-Cladera M, Pérez-Cano FJ, Castellote C, Franch A, Castell M. A diet enriched with cocoa prevents IgE synthesis in a rat allergy model. *Pharmacol Res* 2012;**65**:603–8
11. Saavedra Y, Vergara P. Hypersensitivity to ovalbumin induces chronic intestinal dysmotility and increases the number of intestinal mast cells. *Neurogastroenterol Motil* 2005;**17**:112–22
12. Khodoun M, Strait R, Orekov T, Hogan S, Hogan S, Karasuyama H, Herbert DR, Köhl J, Finkelman FD. Peanuts can contribute to anaphylactic shock by activating complement. *J Allergy Clin Immunol* 2009;**123**:342–51
13. Silver R, Curley JP. Mast cells on the mind: new insights and opportunities. *Trends Neurosci* 2013;**36**:513–21
14. Tonelli LH, Katz M, Kovacsics CE, Gould TD, Joppy B, Hoshino A, Hoffman G, Komarow H, Postolache TT. Allergic rhinitis induces anxiety-like behavior and altered social interaction in rodents. *Brain Behav Immun* 2009;**23**:784–93
15. Li XM, Serebrisky D, Lee SY, Huang CK, Bardina L, Schofield BH, Stanley JS, Burks AW, Bannon GA, Sampson HA. A murine model of peanut anaphylaxis: T- and B-cell responses to a major peanut allergen mimic human responses. *J Allergy Clin Immunol* 2000;**106**:150–8
16. Basso AS, Pinto FA, Russo M, Britto LR, de Sá-Rocha LC, Palermo Neto J. Neural correlates of IgE-mediated food allergy. *J Neuroimmunol* 2003;**140**:69–77
17. Costa-Pinto FA, Basso AS, De Sá-Rocha LC, Britto LR, Russo M, Palermo-Neto JJ. Neural correlates of IgE-mediated allergy. *Ann N Y Acad Sci* 2006;**1088**:116–31

(Received July 9, 2014, Accepted December 30, 2014)