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European Journal of Pharmacology 472 (2003) 221–227
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Systemic anaphylaxis is prevented in alloxan-diabetic rats by a mechanism dependent on glucocorticoids

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Received 6 January 2003; received in revised form 21 May 2003; accepted 26 May 2003

Abstract

This study was undertaken to examine whether glucocorticoids could be implicated in the hyporesponsiveness of diabetic rats to systemic anaphylaxis. Rats were actively sensitized with a mixture of $Al(OH)_3$ plus ovalbumin and challenged i.v. with ovalbumin 14 days later. Diabetes was induced by alloxan-injected i.v. either before or after sensitization. Elevation of total and specific serum immunoglobulin E (IgE) was abolished in rats turned diabetic and then sensitised, but not in those first sensitised and then turned diabetic. In both conditions, increased serum corticosterone levels occurred in parallel with protection of diabetic animals against fatal shock, intestinal haemorrhage and elevation in plasma histamine levels evoked by antigen challenge. The resistance of diabetic rats to fatal shock was no longer significantly different from that of non-diabetic rats following treatment with the glucocorticoid receptor antagonist RU 486 (mifepristone). These findings indicate that endogenous glucocorticoid plays a pivotal role in the phenomenon of hyporeactivity to systemic anaphylaxis in alloxan-diabetic rats.

Keywords: Diabetes; Systemic anaphylaxis; Glucocorticoid; Mast cell

1. Introduction

It has been demonstrated that diabetic patients present many functional abnormalities which can be partially responsible for their failure in mounting a plain inflammatory response (Garcia-Leme, 1989). In line with this concept, some authors reported that the concurrent occurrence of allergic disorders and type 1 diabetes is markedly reduced when compared to the incidence of each one alone (Huang, 1999; Olesen et al., 2001). This observation was made many years ago but the precise mechanism is not yet understood. The possibility does exist that the resistance to allergic provocation results from an imbalance in the T-helper 1/T-helper 2 (Th1/Th2) response. Since autoimmune type 1 diabetes is Th1-dependent and allergy is Th2-dependent, the susceptibility to one disease might lead a state of refractoriness to the other (Huang, 1999).

Systemic anaphylaxis is an acute and often life-threatening reaction which results from the immediate release of pro-inflammatory substances when the allergen interacts with immunoglobulin E (IgE) antibodies anchored on the surface of mast cells or basophils (Busse and Lemanske, 2001; Kay, 2001). Earlier studies demonstrated that alloxan-diabetic mice were resistant to anaphylactic shock (Thompson, 1961; Ganley, 1962; Dhar et al., 1967; Ptak et al., 1983), in a mechanism clearly reversed by treatment with insulin (Thompson, 1961; Ganley, 1962). Interestingly, insulin administration before antigen challenge increased the mortality from anaphylactic shock in actively sensitized rodents (Ptak et al., 1983). One potential explanation for this phenomenon is that the resistance of diabetic animals to anaphylactic reactions is due to an excess of glucose in the blood circulation. Indeed, a marked protection was noted under the condition of hyperglycaemia induced by a massive dose of glucose administered before antigen provocation (Ptak et al., 1983). Another possibility is that insulin depletion may prevent the triggering of IgE-producing cells or decrease the rate of IgE production, which would be associated with T or B cell defects. In fact, the

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doi:10.1016/S0014-2999(03)01934-4

formation of IgE antibodies to sensitizing antigens appeared dramatically reduced in alloxan-diabetic mice (Ptak et al., 1983), suggesting a causative relationship between both phenomena.

In more recent studies, we have reported that sensitised rats turned diabetic after alloxan treatment exhibited a reduced antigen-induced protein extravasation and leukocyte infiltration in the pleural space. Suppression of allergen-evoked pleurisy appeared correlated with a decrease in local mast cell population (Diaz et al., 1996) and an increase in serum corticosterone levels (Diaz et al., 2001). Glucocorticoid hormones have a profound down-regulatory effect on mast cell number and reactivity (for review, see Barnes, 1997). It is noteworthy that elevation in the serum glucocorticoid concentration has already been detected in diabetic patients (Cameron et al., 1984; Roy et al., 1990, 1991). In the current study, we investigated the influence of glucocorticoid hormones on the suppression of anaphylactic shock in alloxinated rats. We found that animals turned diabetic and then sensitized failed to produce specific IgE and did not develop anaphylactic shock. On the other hand, animals first sensitised and then turned diabetic presented intact IgE production and were also resistant to anaphylaxis. In both protocols, the resistance of diabetic rats to allergen challenge occurred in parallel with the elevation in the corticosterone serum levels and was prevented by treatment with the steroid receptor antagonist RU 486 (mifepristone), strongly implicating glucocorticoids in this protective phenomenon.

2. Materials and methods

2.1. Animals, sensitization process and antigenic challenge

Male Wistar rats (180–200 g) were obtained from the Oswaldo Cruz Foundation breeding. All the procedures involving care and use of laboratory animals in this study were examined and approved by the Animal Ethics Committee of the Oswaldo Cruz Foundation (CEUA-FIOCRUZ, Prot. 00085-02). Rats were sensitized with a subcutaneous injection of a mixture containing 50 μg of ovalbumin plus 5 mg of $Al(OH)_3$. Fourteen days later, anaesthetized (pentobarbital, 40 mg/kg, i.p.) animals were challenged by means of an intravenous injection of ovalbumin (3 mg/kg) dissolved in sterile NaCl 0.9% solution (saline). Mortality rate was recorded during 24 h after the allergen challenge.

2.2. Diabetes induction

Diabetes was induced by a single injection of alloxan (40 mg/kg, i.v.), diluted with sterile saline into 12 h-fasted anaesthetized rats, 3 days after or 11 days before active sensitization. Blood glycaemia was determined by means of a glucose monitor in samples obtained from the tail vein. Only rats with glucose levels above 200 mg/dl were considered in further experiments.

2.3. Blood leukocyte counts

The blood was obtained from the tail vein of rats after diabetes induction. Total leukocyte number was determined in Neubauer chamber by means of an optical microscope after dilution of blood samples with 2% acetic acid solution and the differential analysis performed in blood smears stained with May-Grunwald–Giemsa dye.

2.4. Haemoglobin quantification

In order to evaluate the intestinal hemorrhage, tissue haemoglobin content was measured according to the haemiglobicyanide method described by Van Kampen and Zijlstra (1961). Fourteen days after sensitization, ileum fragments (1 g) were obtained from anaesthetized diabetic and non-diabetic rats, 1 h after challenge. Tissue fragments (1 g) were placed in tubes containing Drabkin solution (8 ml) and kept under light protection for 24 h. The final reaction was evaluated spectrofluorimetrically at 540 nm.

2.5. Quantification of plasma histamine levels and of histamine secreted from tissue fragments stimulated with antigen in vitro

Plasma histamine analysis was performed by means of radioenzimatic assay as described by Correa and Saavedra (1981). Blood was obtained through cardiac puncture and mixed with heparin (10 Ui/ml; 1 vol for 9 vol of blood), 1 h after challenge. Plasma was obtained by centrifugation of blood at $270 \times g$ for 15 min. Antigen-shocked animals were pretreated with the histamine and 5-HT receptor antagonist cyproheptadine (5 mg/kg, i.p.) in order to permit their bleeding. Briefly, 10 μl of plasma samples or the standard histamine solution were incubated with 50 μl of a freshly prepared mixture containing histamine-*N*-methyl-transferase, 0.04 μCi of *S*-adenosyl [methyl-^{3}H]methionine and 0.05 M sodium phosphate buffer, pH 7.9. Blanks were prepared by replacing the sample test by sodium phosphate buffer. After incubation overnight at 4 °C, [^{3}H]-methyl-histamine was then extracted and radioactivity counted by means of a Beckman LS-6500 Scintilograph. For evaluation of tissue histamine secretion, ileum segments were removed from diabetic and non-diabetic sensitized rats and washed with Tyrode solution. Small fragments were placed in 24-well tissue culture plates containing Hank's balanced salt solution (HBSS)/Ca^{2+}/Mg^{2+} and then challenged with ovalbumin (0.4 mg/ml) for 15 min. The samples were collected, added to 0.8 N perchloric acid, and after centrifugation at $433 \times g$ for 10 min the supernatant was recovered and stored at −20 °C until histamine fluorimetrical quantification as described by Shore et al. (1959).

Table 1
Influence of diabetes induced by alloxan on the mortality induced by ovalbumin in actively sensitized rats

Group	Dead/Total	Mortality (%)	*P*
Sensitized only	16/20	80	–
Diabetic + sensitized	0/10	0	< 0.001
Sensitized + diabetic	11/30	36	< 0.05

Mortality was evaluated from 0 to 24 h after antigen challenge (ovalbumin, 3 mg/kg).

2.6. Surgical bilateral adrenalectomy

Adrenalectomy was performed through bilateral dorsal incisions in anaesthetized animals. Sham-operated animals were submitted to the same surgical manipulation, but adrenal glands were left intact. Both groups were maintained on a normal diet and allowed free access to 0.9% saline for 7 days.

2.7. Measurement of total serum IgE

Blood was taken by cardiac puncture under light ether anesthesia 14 days after sensitization. After blood coagulation, individual sera were collected and stored at −20 °C until use. Total serum IgE was measured by means of enzyme-linked immunosorbent assays (ELISA) as previously described (Lima et al., 1997). Plates were coated overnight with a mouse IgG1 monoclonal antibody against a heavy chain or rat immunoglobulin (MARE-1) (2 μg/ml) dissolved in carbonate/bicarbonate buffer (0.05 M, pH 9.6) and washed with 10 mM potassium phosphate buffer pH 7.5/0.02% thimerosal/0.05% Tween 20. Nonspecific binding sites were blocked with 0.25 ml 5% PBS skimmed milk at 37 °C for 2 h and the plates washed again. Then, 0.1 ml of serum samples or the standard rat IgE kappa was added to the plates for 2 h. The unbound immunoglobulin was washed off and fixed IgE was recognized after addition of a peroxidase-conjugated mouse IgG1 kappa monoclonal antibody raised against the kappa light chain of rat immunoglobulin (MARK-1) (0.5 mg/ml). After the final washing, plates were developed with K-blue substrate (Neogen, Lexington, KY) for 30 min before stopping the reaction with 0.19 M H_2SO_4 for absorbance reading at 450 nm.

2.8. Quantification of specific IgE

Specific IgE was investigated using passive cutaneous anaphylaxis technique in rats (Ovary, 1964). Serum from 14-day actively sensitized rats was injected intradermally into different region of shaved backs. Ovalbumin and Evans blue dye (25 mg/kg) were co-injected intravenously 24 h later. Over a 30-min period, the animals were killed and the skin sites removed and maintained in formamide solution until complete dye extraction. Evans blue dye leakage was quantified spectrophotometrically at 650 nm as described (Tarayre and Lauressergues, 1979).

2.9. Treatments

Normal rats were treated intraperitoneally with dexamethasone (0.1 mg/kg) and corticosterone (0.5 mg/kg) administered once a day during 3 days before antigen challenge. We also tested the effect of the steroid receptor antagonist RU 486 which was dissolved in 0.5% methylcellulose (v/v in water) and orally injected at 20 mg/kg. The drug was administered into sensitized diabetic rats 2 h before ovalbumin challenge and into diabetic sensitized rats once a day during five consecutive days before antigen. To evaluate the role of vasoactive amines in the anaphylactic intestinal haemorrhage, sensitized rats were treated with histamine and 5-HT receptor antagonist cyproheptadine (5 mg/kg, i.p.), 1 h before stimulation. In control animals, the drugs were replaced by their vehicles.

2.10. Drugs

Alloxan tetrahydrate, corticosterone (21-Hemi-Succinate:BSA), RU 486 (mifepristone), cyproheptadine and *o*-phtaldialdehyde were purchased from Sigma (St. Louis, MO), dexamethasone from Prodome (São Paulo, Brazil), sodium pentobarbital from Rhône Mérieux (Tallaght, Dublin) and ovalbumin from Biochemika Fluka (Switzerland).

2.11. Statistical analysis

The data were statistically analysed by an analysis of variance (ANOVA) followed by the Newman–Keuls–Student's *t*-test. In the case of the lethality rate, the χ^2-test (Fisher's exact test) was used. Probability values of 0.05 or less were considered significant.

3. Results

3.1. Prevention of anaphylactic shock in diabetic rats

The intravenous injection of ovalbumin (3 mg/kg) into actively sensitised rats elicited a severe systemic anaphy-

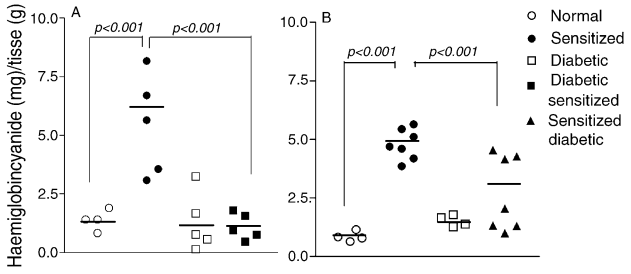

Fig. 1. Influence of diabetes induced by alloxan (40 mg/kg, i.v.) on intestinal hemorrhage induced by ovalbumin (OVA, 3 mg/kg, i.v.) in actively sensitized rats. Diabetes was induced before (A) or after (B) active sensitization and intestinal haemorrhage was evaluated at 30 min after allergen challenge. Values represent the mean ± S.E.M. of at least six animals.

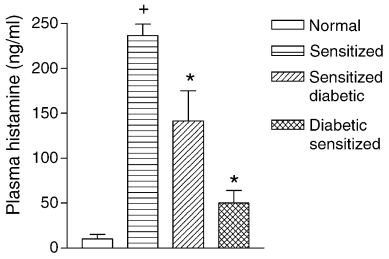

Fig. 2. Influence of diabetes induced by alloxan (40 mg/kg, i.v.), before (diabetic sensitized) or after active sensitization (sensitized diabetic), on increase in plasma histamine levels noted 15 min after ovalbumin (3 mg/kg; i.v.) provocation. Values represent the mean ± S.E.M. of at least six animals. $^{+}P<0.01$ as compared to non-sensitized rats; $^{*}P<0.05$ as compared to sensitized non-diabetic rats.

lactic response with lethality rate between 75% and 100% (Table 1). This phenomenon happened in parallel with a marked intestinal haemorrhage (Fig. 1) and substantial increase in plasma levels of histamine (Fig. 2). Vasoactive amines seem to play a pivotal role in this process since treatment of sensitized rats with the histamine and 5-HT receptor antagonist cyproheptadine, 1 h before challenge, inhibited the intestinal haemorrhage. Values of haemiglobicyanide (mg) per g of tissue were 1.38 ± 0.10 and 6.19 ± 0.80 ($n=5$; $P<0.001$) for sensitized non-challenged and challenged rats, respectively, and 2.13 ± 0.28 ($n=5$; $P<0.001$) for cyproheptadine-treated animals.

Animals turned diabetic before sensitization exhibited substantial reduction in antigen-evoked mortality (Table 1), intestinal haemorrhage (Fig. 1A) and plasma histamine accumulation (Fig. 2). Animals turned diabetic after sensitization also exhibited reduction in the mortality rate (Table 1), intestinal haemorrhage (Fig. 1B) and histamine release into the plasma (Fig. 2), although the magnitude of blockade, in that case, appeared significantly lower as compared to the former group.

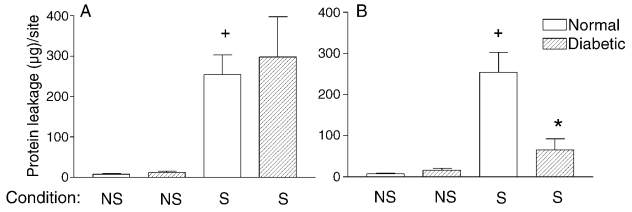

Fig. 3. Influence of diabetes on increase in the levels of serum IgE antibodies produced against ovalbumin in actively sensitized rats (hatched columns), evaluated by passive cutaneous anaphylaxis assay. Diabetes was induced by alloxan (40 mg/kg, i.v.), before (A) or after (B) active sensitization, and serum samples were obtained 14 days after sensitization. Evans blue dye leakage caused by intradermal injection of serum was evaluated 24-h post-sensitization. Serum from non-sensitized rats (open columns) was used as controls. Values represent mean ± S.E.M. of at least five animals. $^{+}P<0.01$ as compared to non-sensitized rats; $^{*}P<0.05$ as compared to sensitized non-diabetic rats.

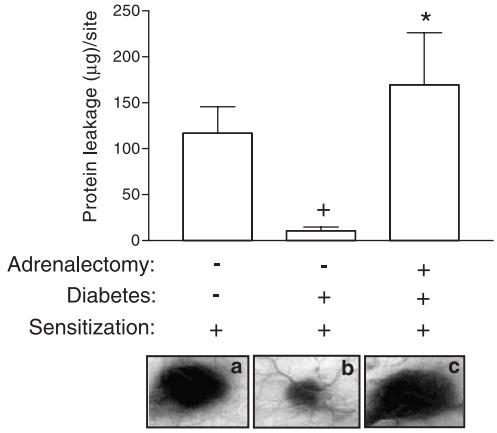

Fig. 4. Influence of endogenous glucocorticoids on suppression of ovalbumin specific IgE antibody formation in diabetic sensitized rats, evaluated by passive cutaneous anaphylaxis assay. Animals were submitted to bilateral adrenalectomy 7 days before diabetes induction (alloxan, 40 mg/kg, i.v.), which was followed by active sensitization procedure. Sera were recovered 14 days after sensitization. Values represent means ± S.E.M. of four animals. $^{+}P<0.01$ as compared to sensitized rats; $^{*}P<0.05$ as compared to sham-operated diabetic sensitized rats. Panels a, b and c show passive cutaneous anaphylaxis responses caused by sera from sensitized, sham-operated diabetic sensitized and adrenalectomized diabetic sensitized rats, respectively.

3.2. Correlation between severity of anaphylaxis reaction and IgE production

According to ELISA measurements, serum levels of total IgE increased from 0.45 ± 0.05 ng/ml (mean ± S.E.M., $n=5$) to 1.28 ± 1 ng/ml in sensitized non-diabetic animals. This increase was abolished in rats turned diabetic before sensitization but remained unaltered in animals in which diabetes was induced after sensitization with values of 0.68 ± 0.06 and 1.24 ± 0.33 ng/ml, respectively. Similar profile was observed concerning specific anti-ovalbumin IgE, as attested by passive cutaneous anaphylaxis reaction. The serum of rats in which diabetes was induced after sensitization caused a passive cutaneous anaphylaxis reaction in an extent comparable to that produced by serum from

Table 2
Effect of treatment with the steroid receptor antagonist RU 486 on the suppression of anaphylactic shock noted in sensitized diabetic and diabetic sensitized rats

Group	RU 486	Dead/Total	Mortality (%)	*P*
Sensitized only	–	9/10	90	–
	+	10/12	83	n.s.
Diabetic + sensitized	–	3/13	23	< 0.001
	+	7/10	70	< 0.001
Sensitized + diabetic	–	5/10	50	< 0.05
	+	17/20	83	< 0.01

Mortality was evaluated from 0 to 24 h after antigen challenge (ovalbumin, 30 mg/kg). RU 486 (20 mg/kg) was orally administered into sensitized diabetic and diabetic sensitized 2 h before antigen provocation and once a day during five consecutive days before antigen.

sensitized non-diabetic animals (Fig. 3A), whereas very faint passive cutaneous anaphylaxis reaction was detected when the serum of rats turned diabetic before sensitization was tested (Fig. 3B). It is noteworthy that when alloxinated rats were submitted to surgical bilateral adrenalectomy, 7 days before diabetes induction, a significant inhibition of the drop in the ovalbumin specific serum IgE concentrations was noted (Fig. 4).

3.3. Involvement of glucocorticoids in diabetes-induced suppression of anaphylactic shock

Measurements of serum corticosterone levels of both diabetic sensitized and sensitized diabetic groups were equally increased as compared to sensitized non-diabetic controls. The values increased from 146 ± 11 ng/ml (mean ± S.E.M., $n = 5$) in controls to 225 ± 13 and 281 ± 40 ng/ml in diabetic sensitized and sensitized diabetic groups, respectively.

The effect of treatment with the glucocorticoid receptor antagonist RU 486 was then investigated. As illustrated in Table 2, RU 486 clearly restored the anaphylactic response in animals subjected to the two diabetogenic regimes. It is noteworthy that administration of either dexamethasone (0.1 mg/kg) or corticosterone (0.5 mg/kg) into sensitized non-diabetic rats during 3 days significantly inhibited antigen-evoked fatal shock, as well as intestinal haemorrhage and augmentation in plasma histamine levels (data not shown). Values of mortality rate reduced from 80% ($n = 20$) in positive control rats to 0% ($n = 12$; $P < 0.001$) and 50% ($n = 11$; $P < 0.01$) in sensitized animals treated with dexamethasone and corticosterone, respectively.

3.4. Influence of diabetic state on the antigen-evoked histamine release from intestinal tissue in vitro

An increase in the levels of histamine released from sensitized ileum segments was obtained after antigen challenge (ovalbumin, 0.4 mg/ml) in vitro. As shown in Fig. 5, the amount of histamine secreted from tissues recovered from both rats sensitized before or after being turned diabetic was clearly reduced as compared to the sensitized non-diabetic control group. In another set of experiments, we demonstrated that administration of dexamethasone to sensitized rats led to suppression of the antigen-evoked histamine release from ileum fragments. Values of histamine (ng) per g of tissue were 44.2 ± 5.6 and 105.6 ± 13.2 ($n = 4$; $P < 0.001$) for sensitized non-challenged and challenged respectively, and 39.8 ± 11.8 ($n = 5$; $P < 0.001$) for tissue of dexamethasone-treated animals.

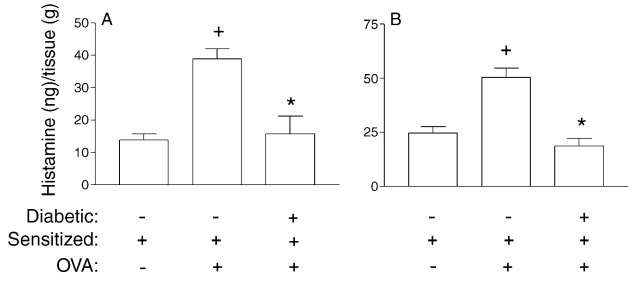

Fig. 5. Influence of diabetes induced by alloxan (40 mg/kg, i.v.), before (diabetic sensitized) (A) or after active sensitization (sensitized diabetic) (B), on ovalbumin (0.4 mg/ml)-induced histamine release from ileum segments in vitro. Values represent the mean ± S.E.M. of least four animals. $^{+}P < 0.01$ as compared to non-challenged tissue; $^{*}P < 0.05$ as compared to ovalbumin-challenged tissue.

4. Discussion

The resistance of diabetic animals to anaphylactic shock was first described in the sixties (Ganley, 1962; Dhar et al., 1967), but its mechanism remains poorly understood. In this study, we readdressed the matter in order to investigate whether glucocorticoid hormones could be implicated in such refractoriness. We found that rats turned diabetic with alloxan and then sensitised, similarly to those sensitized before development of diabetes, were clearly resistant to systemic anaphylactic shock, presenting substantial reduction in the mortality rate, intestinal haemorrhage and plasmatic levels of histamine. While total and specific IgE levels were abrogated in diabetic and then sensitized animals, the IgE levels remained unchanged in animals sensitized and then turned diabetic. In both cases, the suppressive phenomenon was accompanied by significant increase in the amount of serum corticosterone and reversed by treatment with the glucocorticoid receptor antagonist RU 486. In addition, exogenous administration of either dexamethasone or corticosterone clearly protected against the anaphylactic shock, giving support to the interpretation that endogenous glucocorticoids may play a pivotal role in the refractoriness of diabetic animals to systemic anaphylaxis.

Anaphylaxis is a response often explosive in onset. Its symptoms result from action upon one or more target organs, of massive quantities of chemical mediators suddenly released from tissue mast cells and circulating basophils (Metcalfe et al., 1997). Among the possible defined causes of anaphylaxis, the most frequent and best understood mechanism is the IgE-dependent immediate-type hypersensitivity phenomenon (Metcalfe et al., 1997). In the current study, we have shown that normoglycaemic Wistar rats can be actively sensitized to ovalbumin and die of anaphylactic shock when intravenously challenged with this protein 14 days later. These animals exhibited a typical extensive haemorrhagic lesion in the small intestine accompanied by a substantial elevation in the plasma histamine concentration, which reached values approximately 18 times higher than baseline levels. Histamine participation in this process was highlighted by the marked blockade of antigen-evoked intestinal damage following treatment with cyproheptadine. In line with previous studies (Dhar et al., 1967; Ptak et al., 1983), we found that rats

turned diabetic with alloxan and then sensitised to ovalbumin were to a great degree protected from fatal shock, intestinal haemorrhagic lesions and elevation of plasmatic histamine levels.

Diabetes mellitus is a syndrome associated with hyperglycaemia and deficiency in production and secretion of insulin (Bell and Plonsky, 2001). The possibility that the diabetic state can affect the sensitization stage of the anaphylactic reaction has been studied. Clearly, formation of IgE appeared drastically reduced in alloxan-diabetic mice (Ptak et al., 1983). Results of this study also revealed that immune cells recovered from spleen and lymph nodes when transferred from naive into diabetic mice loose their ability to form IgE, whereas the immune cell suspensions from diabetic animals regain the capacity to generate IgE upon transfer to normal recipients. These findings suggest that a soluble factor present in the blood circulation of diabetic mice interferes directly or indirectly with IgE-producing cells. Our data showed that augmentation in total and ovalbumin specific serum IgE levels was completely suppressed in diabetic sensitized rats, a phenomenon which was reversed by adrenalectomy, suggesting that endogenous glucocorticoids could act as down-regulatory factors of the sensitization phase of the anaphylactic reaction. Nevertheless, since both diabetic sensitized and sensitized diabetic animals were resistant to anaphylactic shock, but only the former group presented reduction in IgE levels, we believe that the state of refractoriness under diabetes is not necessarily dependent on the blockade of IgE production.

Hyperactivity of the hypothalamic–pituitary–adrenal axis with consequent hypercortisolism is frequently observed in patients with type 1 and type 2 diabetes (Cameron et al., 1984; Roy et al., 1990, 1998), and seems to be important to the development of some pathologies associated with the disorder. It has been demonstrated that the increased hypothalamic–pituitary–adrenal activity in streptozotocin-induced diabetic rats can be normalized by insulin, in a mechanism related to an up-regulation of glucocorticoid receptor mRNA in the pituitary, allowing glucocorticoid-mediated suppression of adrenocorticotropic hormone (ACTH) secretion (Chan et al., 2001). We hypothesized that an increase in the circulating levels of corticosterone in alloxan-diabetic rats may act to inhibit their reactivity to anaphylactic shock. In fact, we noted that treatment with either dexamethasone or corticosterone clearly reduced antigen-evoked fatal shock in normoglycaemic rats. Moreover, a marked increase in serum corticosterone levels was observed in rats turned diabetics and then sensitized, and also in those sensitized before development of diabetes. If elevation of glucocorticoid levels was indeed crucial to that suppression, the pharmacological blockade of glucocorticoid receptor should restore the sensitivity of diabetic animals. Our findings indicated that treatment with the glucocorticoid receptor antagonist RU 486 indeed restored the reactivity to antigen challenge of both diabetic sensitized and sensitized diabetic rats, reinforcing the view that there was a direct correlation between the long-lasting hypercortisolism associated with diabetes and the refractoriness to anaphylaxis.

Finally, we used intestinal fragments from rats sensitized before and after diabetes induction in order to compare their responsiveness in terms of histamine release following antigen provocation in vitro. We found that there was a complete blockade of antigen-evoked histamine secretion under both conditions, indicating that mast cell number and/or reactivity were affected whatever the protocol used. It is noteworthy that a marked blockade of histamine secretion was also noted when intestinal fragments were obtained from sensitized rats pretreated with dexamethasone in vivo.

Since glucocorticoids are reputed to have suppressive actions upon number and reactivity of tissue mast cells (Barnes, 1997), we propose that the resistance of sensitized rats to anaphylactic shock under diabetic condition is accounted for by elevation of the circulating levels of endogenous glucocorticoid hormones, resulting in the down-regulation of the mast cell response.

Acknowledgements

The authors thank Miss Sabrina Lopes Lucena and Juliane Pereira da Silva for their technical assistance. This study was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundaç ão de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) and PAPES 3/FIOCRUZ Scientific Programme, Brazil.

References

Barnes, P., 1997. Molecular mechanisms of glucocorticoid action in asthma. Pulm. Pharmacol. Ther. 10 (1997), 3–19.

Bell, G.I., Plonsky, K.S., 2001. *Diabetes mellitus* and genetically programmed defects in β-cell function. Nature 414, 788–791.

Busse, W.W., Lemanske, R.F., 2001. Asthma. N. Engl. J. Med. 344, 350–362.

Cameron, O., Kronfol, G.Z., Greden, J.F., Carroll, B.J., 1984. Hypothalamic–pituitary–adrenocortical activity in patients with *Diabetes mellitus*. Arch. Gen. Psychiatry 41, 1090–1095.

Chan, O., Chan, S., Inouye, K., Vranic, M., Matthews, S.G., 2001. Molecular regulation of the hypothalamo–pituitary–adrenal axis in streptozotocin-induced diabetes: effects of insulin treatment. Endocrinology 142, 4872–4879.

Correa, F.M.A., Saavedra, J.M., 1981. Increase in histamine concentrations in discrete hypothalamic nuclei of spontaneously hypertensive rats. Brain Res. 205, 445–451.

Dhar, H.L., Sanyal, R.K., West, G.B., 1967. Anaphylactic shock and the blood sugar level. J. Pharm. Pharmacol. 19, 699–700.

Diaz, B.L., Serra, M.F., Alves, A.C., Pires, A.L.A., Correa, F.M.A., Cordeiro, R.S., Martins, M.A., Silva, P.M.R., 1996. Alloxan diabetes reduces pleural mast cell numbers and the subsequent eosinophil influx induced by allergen in sensitized rats. Int. Arch. Allergy Immunol. 111, 36–43.

Diaz, B., Barreto, E., Cordeiro, R., Perretti, M., Martins, M., Silva, P., 2001. Enhanced serum glucocorticoid levels mediate the reduction of serosal mast cell numbers in diabetic rats. Life Sci. 68, 2925–2932.

Ganley, O.H., 1962. Studies on the prevention of sensitization by *Bordetella pertussis* in alloxan-diabetic mice. Can. J. Biochem. Physiol. 40, 1179–1183.

Garcia-Leme, J., 1989. Hormones, cells and inflammation. In: Garcia-Leme, J. (Ed.), Hormones and Inflammation. CRC Press, Boca Raton, FL, pp. 192–214.

Huang, S., 1999. Asthma and diabetes. Lancet 354, 515–516.

Kay, A.B., 2001. Allergy and allergic diseases. First of two parts. N. Engl. J. Med. 344, 30–37.

Lima, M.C., Prouvost-Danon, A., Silva, P.M.R., Chagas, M.S., Calheiros, A.S., Cordeiro, R.S., Latine, D., Bazin, H., Ryan, U.S., Martins, M.A., 1997. Studies on the mechanisms involved in antigen-evoked pleural inflammation in rats: contribution of IgE and complement. J. Leukoc. Biol. 61, 286–292.

Metcalfe, D.D., Baram, D., Mekori, Y.A., 1997. Mast cells. Physiol. Rev. 77 (1997), 1033–1079.

Olesen, A.B., Juul, S., Birkebaek, N., Thestrup-Pedersen, K., 2001. Association between atopic dermatitis and insulin-dependent diabetes mellitus: a case-control study. Lancet 357, 1749–1752.

Ovary, Z., 1964. Passive cutaneous anaphylaxis. In: Ackroyd, F. (Ed.), Immunological Methods. CIOMS Symposium. Blackwell, Oxford, UK, pp. 59–70.

Ptak, W., Rewicka, M., Strzyzewska, J., Kollat, M., 1983. Alleviation of IgE-mediated immune reactions in hypoinsulinaemic and hyperglycaemic mice. Clin. Exp. Immunol. 52, 54–60.

Roy, M., Collier, B., Roy, A., 1990. Hypothalamic–pituitary–adrenal axis dysregulation among diabetic outpatients. Psychiatry Res. 31, 31–37.

Roy, M., Collier, B., Roy, A., 1991. Dysregulation of the hypothalamo–pituitary–adrenal axis and duration of diabetes. J. Diabetes Its Complicat. 5, 218–220.

Roy, M.S., Roy, A., Brown, S., 1998. Increased urinary-free cortisol outputs in diabetic patients. J. Diabetes Its Complicat. 12, 24–27.

Shore, P.A., Burkhalter, A., Cohn, V.H., 1959. A method for the fluorimetric assay of histamine in tissues. J. Pharmacol. 127, 182–186.

Tarayre, J.P., Lauressergues, H., 1979. Action of some non-steroid antiinflammatory drugs on skin anaphylaxis due to ovalbumin in the rat and the guinea-pig. Arzneim.-Forsch. Drug. Res. 29, 774–776.

Thompson, G.E., 1961. Alloxan and hypersensitivity. Nature 190, 822.

Van Kampen, E.J., Zijlstra, W.G., 1961. Standardization of hemoglobinometry: II. The hemiglobincyanide method. Clin. Chim. Acta 6, 538–544.