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Effect of Experimental Anaphylaxis on certain Biochemical Parameters in Goat

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Abstract: This study was carried out to analyse certain biochemical parameters altering in experimental anaphylaxis in goat. Goats were sensitized to horse serum and were subjected to systemic anaphylactic shock. Blood samples were collected at 24 h after each injection of antigen during sensitization and at 10, 60 and 240 min after antigenic challenge and analysed. Results revealed a significant increase in blood glucose during sensitization and post sensitization. Significant increase in serum sodium and potassium was observed at 10 min post antigenic challenge. Total serum lipids were significantly decreased during sensitization and after challenge. Serum total proteins were decreased significantly at the last injection of sensitization and 10 min after challenge. It is concluded that anaphylaxis induces blood biochemical changes in goats and suggested the development of disorders in cardiovascular and respiratory system in goat specie.

Key words: Anaphylaxis, biochemical, experimental, sensitization, endotoxin shock lipopolysaccharides

INTRODUCTION

Anaphylaxis refers to a severe allergic reaction in which prominent dermal and systemic signs and symptoms manifest[1]. It involves prior sensitization to an allergen with later re-exposure, producing symptoms via an immunologic mechanism. This mechanism involves release of mediators, which include histamine, leukotriene C4, prostaglandin D2 and tryptase[1,2].

The physiologic responses to these mediators leads to the classic symptoms of anaphylaxis; including flushing; urticaria; pruritus; bronchospasm and increased vascular permeability and stimulation of sensory nerve endings. Hypotension and shock can result from intravascular volume loss, vasodilation and myocardial dysfunction[1-3].

The incidence of anaphylactic reactions seems to be increasing and perhaps the rise is due to increased environmental and medical exposure to agents such as foods, drugs, other biologicals and insect venoms[3]. Sensitivity to anaphylactic shock or endotoxic shock is largely of specie dependant[4].

Experimental anaphylaxis produces characteristic biochemical changes and investigated in guinea pig, rabbit, mouse, dog, calf and horse. Little research has been done on these lines in goat specie in contrast to the study done in other ruminants and large animals[5-7]. Primary purpose of present study was to analyze certain biochemical parameters altering in experimental anaphylaxis in goat.

MATERIALS AND METHODS

The study was conducted during July 2002 to October 2002, at Department of Veterinary Pharmacology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam.

Six visibly normal goats of mixed breed under one year of age and weighing 18 kg (average) were used. Animals were allowed to acclimatize for 7 days during which they were kept on concentrates and green and fresh grasses. Serum was obtained from freshly collected blood of goats and biochemical parameters i.e., glucose, sodium, potassium, total lipids and total proteins were recorded to establish base line values. After obtaining control values all animals were used for experiments.

Sensitization: Horse serum was used as antigen. Serum was obtained from blood of the healthy horse. All animals were sensitized by injecting horse serum I/V at the dose of 0.2 mL kg^{-1} body weight. One hour after I/V injection an additional subcutaneous injection of the same dose of antigen was given in the neck region. Two more subcutaneous injections of the same dose (0.2 mL kg^{-1} body weight) of horse serum obtained from freshly collected blood were injected at weekly intervals i.e., 7 and 14 days after the first injection. Blood samples were collected from goats after 24 h of each injection; serum was obtained and analysed for possible changes in biochemical. After the last injection, three more weeks

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were allowed (latent period) before the animals were challenged to induce anaphylaxis.

Systemic Anaphylaxis: After three weeks of sensitization, all the animals were challenged with the same dose (0.2 mL kg^{-1}) of horse serum administered intravenously. Serum was obtained from goat blood collected at 10, 60 and 240 min post antigenic challenge and analysed for alteration in biochemical values.

Biochemical tests: Glucose was determined by GOD-PAP method using Human kit[8].

Sodium was carried out with colorimetric method using sodium reagent set[9] .

Potassium rapid kit (Ingo test, Ingelheim) was used for photometric determination of potassium in serum[10]

Total serum Lipid was measured by using Merko test 3321 kit[11].

Total protein was determined by Biuret method using Merko test kit[12].

A two way analysis of variance was used to compare the differences between control and horse serum responses during sensitization and post challenge.

RESULTS

Blood samples were collected at 24 h after each injection of antigen during the sensitization and at 10, 60 min and 4 h after antigenic challenge. The values were compared with their respective controls.

During the process of sensitization blood glucose values obtained after third injection of antigen showed significant ($p<0.05$) increase from control (Table 1). However significant increase could be detected at 10 and 60 min after challenge (Table 2).

Sodium appeared to increase after first, second and third antigenic injection during sensitization (Table 1) although the values did not reach the statistical significance ($p<0.05$). However, during systemic anaphylaxis a significant increase in sodium was observed at 10 min post antigenic challenge (Table 2).

Analysis of data after the second and third injection of antigen during sensitization revealed a non significant decrease in potassium (Table 1), where as after challenge, significant increase was observed at 10 min when compared to controls (Table 2).

During the process of sensitization, the second and third antigenic administration caused significant ($p<0.05$) decrease in total lipids (Table 1), where as total lipid content significantly ($p<0.05$) decreased at 60 and 240 min post antigenic challenge when compared with control (Table 2).

The total protein content of blood appeared to increase after the first and second dose of antigen during sensitization but significant ($p<0.05$) increase occurred only after third dose (Table 1). On the other hand challenge dose caused a significant ($p<0.05$) increase in total protein at 10 min (Table 2).

DISCUSSION

Experimentally the anaphylactic reactions have been induced by sensitizing animals to foreign proteins and exposing them to the same antigen after 2-3 weeks. On re-exposure to antigen in sufficient quantity, the antigen interacts with the cell fixed antibodies leading to the degranulation of mast cells and basophils causing release of anaphylactic mediators which are responsible for the anaphylactic reactions[3, 13-15].

This study confirms that during anaphylaxis broad range of changes occur in blood biochemistry.

In this study the success of sensitization was confirmed by response to 2 and 3 antigenic injections suggesting the gradual rise in antibodies. During

Table 1: Biochemical values of 6 goats during sensitization to horse serum. Samples were taken at post first injection, post second injection and post third injection

	Control	Post1st injection	Post 2nd injection	Post 3rd injection
Glucose (mg dL^{-1})	54.93±05.61	54.21±3.15	53.17±3.24	73.29±2.46*
Sodium (m Eq L^{-1})	157.29±04.29	163.83±5.09	165.50±5.12	162. 60±10.73
Potassium (m Eq L^{-1})	06.49±0.46	06.90±0.89	05.81±02	05.95±0.10
Total lipids (mg dL^{-1})	356.25±4.75	352.17±10.75	251.00±10.75*	266.50±7.15*
Total Protein (g dL^{-1})	06.90±0.51	07.31±0.22	07.86±0.67	10.26±0.56*

Data is expressed as Mean±SD, *Significantly ($p<0.05$) different from control

Table 2: Biochemical values of 6 goats after challenge to horse serum. Samples were taken at 10, 60 and 240 min

	Control	After 10 min of injection	After 60 min of injection	After 240 min of injection
Glucose (mg dL^{-1})	66.88±03.48	90.33±10.15*	89.17±03.24*	70.29±12.46
Sodium (m Eq L^{-1})	162.83±04.10	208.00±06.09*	166.00±15.12	168.33±07.73
Potassium (m Eq L^{-1})	05.43±00.26	07.92±00.44*	05.83±01.34	05.61±00.87
Total lipids (mg dL^{-1})	293.37±12.75	280.17±09.75	208.17±10.75*	215.67±17.15*
Total Protein (g dL^{-1})	07.12±00.76	09.11±00.37*	07.01±00.67	07.19±00.66

Data is expressed as Mean±SD, *Significantly ($p<0.05$) different from control

sensitization antigenic exposure caused significant effect on blood sugar, total proteins and lipids and potassium (Table 1). This is in agreement with others reporting gradual development of response to repeated antigenic administration during the process of sensitization[2,16,17]. Production of anaphylactic antibodies by large single or multiple doses of antigen has been reported and it was found that repeated injections of minute amounts of antigen were more effective[3,18].

As shown in Table 1 the serum glucose increased significantly ($p<0.05$) after third injection of antigen during sensitization. Similarly significant ($p<0.05$) increase was observed at 10 and 60 min after antigenic challenge (Table 2). These results are in agreement with several workers who reported that stress and excitement causes an increase in blood glucose in cattle[19,20]. Hyperglycaemia has also been reported in goats when they were induced lipopolysaccharide (LPS) shock[7]. Alteration in glucose homeostasis is a prominent feature of endotoxima and endotoxins are capable of inducing anaphylactic hyperglycaemia in cattle and lambs[5,6,21]. During anaphylactic stress the sympathetic activity is increased which in turn releases the increased adrenaline. Increased secretion from adrenal cortex increases the blood glucose[22,23]; same may be the case with goat species for increased glucose level during anaphylaxis.

In this study the serum potassium level was not altered significantly during the process of sensitization (Table 1), where as it was significantly increased at 10 min after challenge (Table 2). Experimental anaphylaxis and infection induced increased potassium level were also reported in calves[20,24]. Abnormal level of serum potassium may be associated with many functional and structural abnormalities including abnormalities, of the myocardium, neuromuscular system and disturbances in carbohydrate tolerance or in disease condition[25]. Contraction of smooth muscle is a prominent feature of anaphylaxis and increased potassium level can induces the contraction of smooth muscle[26]. Increase of serum potassium level may be the efflux from vascular smooth muscles occurring during contraction and permeability changes or may be release of ion from mast cell etc.[27].

No significant alteration was observed in sodium ions during sensitization, which confirms the findings in calves[27], in horses[24] and in dogs[28]. These workers could not find any appreciable change in sodium ions during sensitization. After challenge we have observed a significant ($p<0.05$) increase in sodium at 10 min which then return to normal (Table 2). The sodium helps in the preservation of the cell permeability and during anaphylaxis capillary permeability is increased. During increased permeability, monovalent ions like Na, Cl and HCO_3 diffuses more rapidly[29]. During anaphylaxis the decreased pressure causes a decrease in osmotic pressure of blood, thus both increased permeability and decreased osmotic pressure may cause an increased sodium level in blood immediately after challenge. In contrast to above findings, a significant decrease in sodium level was observed in goats when they were experimentally shocked with endotoxin[6].

Total lipid decreases significantly during sensitization and post antigenic challenge (Table 1 and 2). This is in agreement with the finding of Singh and Sodhi[30], who observed hypercholesterolemia in calves with *E. coli* endotoxins. In contrast to this, endotoxin induced hypercholesterolaemia was observed in lambs[31]. On the other hand, in calves, no alteration in total serum lipid was observed with endotoxin shock[21,23] this suggests an important specie difference. It is reported that endotoxin induces changes in lipid metabolism which are dose dependant[32]. One possibility of decreased total lipid content in anaphylaxis is that, increased capillary permeability may facilitate the diffusion of the fine emulsified fat droplets through the capillary wall causing an over all decrease in total lipid content.

Total protein in this study appeared to increase after the first and second dose of antigen during sensitization but revealed statistical significance ($p<0.05$) only after third dose (Table 1). Similarly antigenic challenge caused the same hyperprotienemia that was significant only at 10 min (Table 2). Coles[33] reported that hyperprotienemia may occur in any kind of shock. Shock induced increase in serum total protein was also observed in dogs[28] and in guinea pig[34]. On the other hand, Nagaraja *et al.*[23] reported that total protein was slightly lower in calves injected with endotoxin, probably because of increased serum albumin in milk whey. Similarly experimental endotoxic shock did not alter the total protein level in horses[35] in lambs[31] and in calves[20,30].

It is concluded that hypersensitivity reaction induces many blood biochemical changes that characterize the anaphylaxis in goat. Such changes may be attributed to involvement of cardiovascular and respiratory system and contribute to the development of disorder of these organs. Changes observed are helpful in making the diagnosis of anaphylaxis and are important from therapeutic standpoint.

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