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Anaphylactic death: the effect of aminoguanidine and heparin on histamine and stress hormones in guinea pigs

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Summary. The modifying effect of aminoguanidine (a histaminase inhibitor) and heparin (a histaminase liberator) on anaphylactic shock in guinea pigs was studied using ovalbumin as an antigen and trigger. The animals died of the shock, the time to death remaining unaltered by the drugs. Serum histamine and cortisol values were high after shock, but were reduced by heparin. Both noradrenaline and adrenaline in plasma were also elevated after shock, the final concentration of the latter being lowered by heparin. The lungs were dilated, indicating bronchoconstriction. The results confirm the role of histamine in anaphylactic shock and its potential value for the diagnosis in this kind of rapid death, in which morphological signs are scarce or lacking. Its diagnostic value still requires confirmation, however, which only autopsy studies can supply. It also appears that pretreatment of the animals with heparin affected the blood cortisol and catecholamines, which are involved in the shock mechanism as countermeasures, although aminoguanidine did not have any effect.

Key words: Anaphylactic death – Sudden death, anaphylactic shock

Zusammenfassung. Es wurde der Einfluß von Aminoguanidin (Histaminaseinhibitor) und Heparin (Histaminaseliberator) auf den durch Ovalbumin induzierten anaphylaktischen Schock an Meerschweinchen untersucht. Die Tiere starben unbeeinflußt von Medikamenten am Schock. Die Serumhistamin- und -cortisolspiegel waren nach dem Schock erhöht, wurden aber durch Heparin gesenkt. Noradrenalin and Adrenalin waren im Plasma ebenfalls erhöht, die Endkonzentration des letzteren wurde durch Heparin gesenkt. Die Lungen waren als Zeichen des Bronchospasmus überbläht. Die Ergebnisse unterstreichen die Rolle des Histamin beim anaphylaktischen Schock und seinen potentiellen Wert für die Beurteilung plötzlicher Todesfälle, bei denen morphologische Veränderungen fehlen oder spärlich sind. Die diagnostische Bedeutung bedarf noch der Bestätigung durch Autopsiestudien. Die

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Vorbehandlung der Versuchstiere mit Heparin betrifft die Stresshormone, die im Mechanismus des Schocks eine Gegenrolle spielen. Aminoguanidin hat dagegen keinen Einfluß.

Schlüsselwörter: Schock – Plötzlicher Tod, anaphylaktischer Schock

Introduction

Anaphylactic reaction is one of the causes of death that are difficult to determine because of a lack of any distinct morphological signs upon which a diagnosis can be based. The mechanism of death is thought to be mainly histamine shock with hypotension and/or bronchoconstriction. Other intermediates, such as leukotrienes, are also involved in the later stages of the shock [1].

A report on 43 cases of anaphylactic death mentions such pathologic changes as laryngeal edema, pulmonary emphysema and edema, and intra-alveolar hemorrhages. Most of the victims had died within 30 min of exposure, which explains the scarcity of morphological findings. The pulmonary changes were also considered nonspecific [2].

This present state of the art calls for a search for markers of anaphylactic reaction that appear rapidly in the blood, for instance, and can be demonstrated post mortem. Morphological signs demonstrable by feasible methods are not likely to be present in patients with an allergic disposition who do not have other chronic illnesses. Eosinophilia in the spleen has been suggested as a marker of an allergic disposition, but this needs more confirmation [3].

We have found elevated histamine values in cases of anaphylactic death, and this led us to study the possibilities for improving the diagnostic criteria for this type of death. One experiment showed high histamine values in guinea pigs that died rapidly of albumin anaphylaxis, but not in those animals in which death took 30 min [4]. The delay was considered to be an effect of histaminase, which inactivated the histamine liberated into the blood more slowly after intraperitoneal injection of the antigen than after intracardial injection. Aminoguanidine is an inhibitor of histaminase [5] and thus might be expected to affect the outcome of anaphylactic shock, making it more rapid and lethal more often. Heparin is a liberator of histaminase in guinea pigs [6] and should therefore prevent or alleviate the symptoms of anaphylactic reaction.

The aim of this study was to test the effect of aminoguanidine and heparin on the outcome of anaphylactic shock and to analyze the changes in the concentrations of the hormones involved in the mechanism of the fatal shock and countermeasures to it. Attention was also paid to morphological changes in the lungs.

Materials and methods

Adult guinea pigs of both sexes and weighing 600–900 g were housed in groups of ten and maintained on a 12-h light-dark schedule. They were sensitized with 100 mg ovalbumin given intraperitoneally 2 months before the actual experiment. For the experiment the animals were divided into four groups. Those in group I (*n;* 8 + 3) received 400 mg ovalbumin (triggering dose) (Sigma, St. Louis, USA) in a 0.9% NaCl solution i.p.; those in group II (*n;* 7 + 3) were

given aminoguanidine bicarbonate (Sigma) 10 mg i.p. 10 min before injection of the same dose of albumin; group III animals (*n;* 7 + 3) received 1000 IU heparin i.m. 15 min before injection of the albumin; and those in group IV (*n;* 7 + 3), which received a 0.9% NaCl solution, served as controls, being killed with CO_2 after the same length of time as the death struggle lasted in the experimental animals.

Records were kept of the symptoms of anaphylactic shock induced by the albumin and the moment of death. At the moment of death the thorax was cut open and blood rapidly collected by heart puncture for centrifugation.

Attempt at postmortem analysis

Three guinea pigs from each group were left in a cold room (+4°C) for 3 days and no samples were taken at death. We attempted to take blood samples on the 3rd postmortem day, but the blood had completely coagulated and dried during the postmortem period and no usable samples could be obtained for the analyses.

Every animal was autopsied and the appearance of the lungs noted. A piece of lung was fixed in 10% neutral formalin and the sections stained with hematoxylin and eosin.

Measurement of histamine and cortisol

Histamine in serum was determined by a modified fluorometric method [7]. Histamine was selectively extracted into isoamyl alcohol under the proper conditions of K_2HPO_4 (3.5–4.0 g) and pH (8.8–8.9), and the fluorescence of the fluorophor histamine orthophthaldialdehyde was measured in the presence of citric acid using an Aminco-Bowman spectrophotofluorometer (excitation at 358 μm and emission at 446 μm). A 70% recovery of histamine from the serum was achieved.

Serum cortisol was also measured by a fluorometric method [8] involving double extraction with methyl dichloride and measurement of fluorescence by Aminco-Bowman spectrophotofluorometer at 530 μm with activation at 470 μm 8 and 16 min after extraction into the fluorescence reagent (H_2SO_4 and ethanol, 3:1).

Catecholamine sampling and analysis

The blood samples were taken into conical polypropylene tubes containing 20 μl 0.1 *M* Na_2 EDTA and 0.01 *M* $Na_2S_2O_5$. The plasma was separated out by centrifugation and stored at −70°C until analyzed.

The catecholamines were extracted from the plasma with aluminum oxide [9], and noradrenaline and adrenaline concentrations were measured by high-pressure liquid chromatography using an electrochemical detector [10].

The results were evaluated statistically using an analysis of variance and the Bonferroni test.

Results

Symptoms of anaphylactic shock and time of death

The guinea pigs lay down 5–15 min after the injection of albumin and began twiching their noses and jerking their limbs. Death ensued an average of 44 min after injection in group I. One animal did not die after this injection, but a second injection killed it in 36 min. The mean time of death in group II (aminoguanidine) was 40 min. One of the ten guinea pigs died after only 5 min, and two guinea pigs did not die from the albumin injection and were killed with CO_2. In group III (heparin) death occurred after an average of 39 min. One animal in

this group died 5 min after the injection and two others did not react to the triggering dose and were killed with CO_2.

Serum histamine and cortisol

Serum *histamine* values are presented in Fig. 1. The average concentration was significantly ($P < 0.05$) higher in those animals which received albumin and NaCl than in the controls (0.37 ± 0.24 vs 0.12 ± 0.04 µg/ml), and that in the group which received heparin and albumin lower ($P < 0.01$) than that in the albumin group (0.04 ± 0.02 vs 0.37 ± 0.24 µg/ml). The concentration in the group which received aminoguanidine and albumin did not differ significantly from either the albumin or the control group, but was slightly higher than in the control group (0.21 ± 0.07 vs 0.12 ± 0.04 µg/ml).

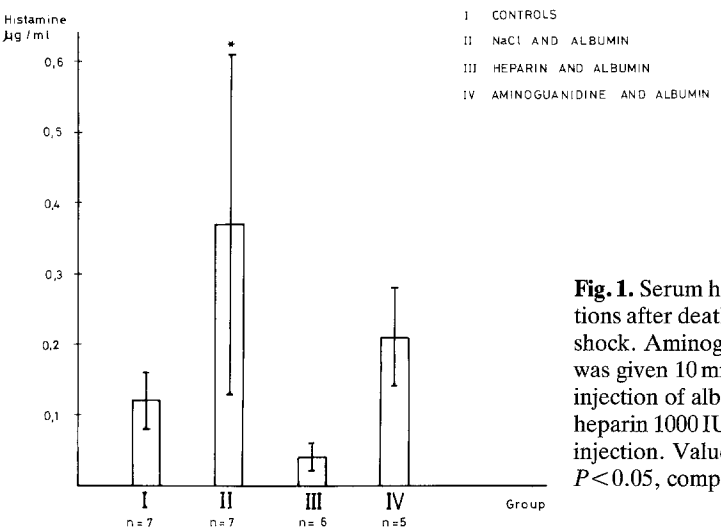

Fig. 1. Serum histamine concentrations after death from anaphylactic shock. Aminoguanidine 10 mg was given 10 min prior to the injection of albumin (400 mg) and heparin 1000 IU 15 min prior to the injection. Values are means ± SD. $P < 0.05$, compared to control

Table 1. Serum cortisol concentrations after death from anaphylactic shock (means ± SD)

Group	Cortisol (µg/100 ml)
I NaCl and albumin ($n = 7$)	147.9 ± 32.1***, +
II Aminoguanidine and albumin ($n = 5$)	122.8 ± 34.6**
III Heparin and albumin ($n = 6$)	103.2 ± 42.1**
IV Controls ($n = 7$)	40.5 ± 31.1

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared with the controls

+$P < 0.05$ compared with group III

Table 2. Plasma noradrenaline (NA) and adrenaline (A) concentration in guinea pigs after death from anaphylactic shock (means ± SD)

Groups	NA (ng/ml)	A (ng/ml)
I NaCl and albumin ($n = 7$)	133.60 ± 41.32*	164.29 ± 37.61**, +
II Aminoguanidine and albumin ($n = 7$)	154.64 ± 46.55**	180.11 ± 91.79**, +
III Heparin and albumin ($n = 5$)	132.84 ± 43.98*	77.50 ± 41.89
IV Controls ($n = 6$)	78.22 ± 14.41	61.85 ± 25.31

*$P < 0.05$; **$P < 0.01$ compared with controls
+$P < 0.05$ compared with group III

Anaphylactic shock increased the average amount of *cortisol* in the serum in every experimental group over that in the controls (Table 1). Aminoguanidine did not significantly alter the cortisol content, whereas heparin reduced it significantly ($P < 0.05$) compared with the albumin group.

Noradrenaline and adrenaline in plasma

The mean *noradrenaline* concentration was significantly elevated at death in all groups that had suffered anaphylactic shock, to about twice the control value (Table 2). Thus aminoguanidine and heparin had no modifying effect on the NA response.

The mean *adrenaline* value was about three times the control value in the albumin and aminoguanidine groups, but no significant rise was observed when heparin had been given. Thus, this group differed significantly from the other two groups that suffered anaphylactic shock.

Histological observations in the lungs

A common observation in all the groups that had suffered anaphylactic shock was acute dilatation of the lungs. Granulomatous inflammation was seen in a few animals in all groups, and this was considered to be a "normal" phenomenon. Eosinophilia of the bronchus wall was not seen in any group.

Discussion

The fact that the time to death from anaphylactic shock was not significantly altered by aminoguanidine or heparin undermined the hypothesis that aminoguanidine, being a histaminase inhibitor, would shorten the time and heparin, a histaminase liberator, would lengthen it. The cause remains unclear, but may have been related to the doses of the drugs. Aminoguanidine also did not significantly increase the histamine concentration at death, only a slightly greater

value than in controls being found after this drug. The value was slightly lower than in the untreated anaphylaxis group. The lowest histamine concentrations were measured after heparin treatment, supporting the idea of increased activity of histaminase, since the time to death in shock was equally long. When these data were considered the conclusion emerged that a low histamine concentration in the serum does not necessarily exclude anaphylactic reaction.

Other substances besides histamine are released during anaphylactic reaction and can cause death. One of these is platelet activating factor (PAF), which is a phospholipid liberated from tissues and is known to cause both bronchoconstriction and a rapid decrease in arterial pressure in rabbits, either of which may lead to of death [11]. The effect of this factor in guinea pigs is bronchoconstriction [12]. Possible mediators of the effects of platelet activating factor are thromboxane A_2, Ca ions or other unknown bronchoconstrictor substances released from the platelets.

Leukotrienes (LTC) are known to be mediators of anaphylactic reactions; they are derivatives of arachidonic acid released from polymorphonuclear leukocytes. They are slow-reacting substances, which also cause bronchoconstriction and reduce cardiac contraction. LTC_4 was found to be 100 times as potent as histamine in causing an increase in insufflation pressure in guinea pigs [1]. The agonal time observed in our experiments, about 30 min, fits with the role of LTC.

The most striking morphological finding in our guinea pigs was "acute emphysema" due to bronchoconstriction, and thus one mechanism of death may be assumed to have been asphyxiation. On the basis of our earlier observations, the present results and other cited reports concerning guinea pigs, the most important factors in the mechanism seem to be histamine in the acute phase and platelet activating factor and LTC in the later phase. The role of the different anaphylaxis mediators seems also to be dependent on the route of administration of the challenging antigen. If it is given intracardially death follows rapidly and is probably due to histamine, but if the antigen is given i.p. or i.m. the agony lasts about 30 min and the other mediators probably play a more decisive role.

The survival rate for guinea pigs immunized and challenged with ovalbumin was improved from 0 to 64% by pretreatment with a combination of diphenhydramine, BW-755C (a cyclo-oxygenase and lipoxygenase synthesis inhibitor) and a PAF receptor antagonist, either kadsurerone or alphrazolam [13], which support the role of several mediators in the mechanism of anaphylactic shock.

Catecholamines (CA) are physiological antagonists of histamine, preventing its release in anaphylactic shock, and thus act as "physiological drugs," alleviating the bronchoconstriction. Elevated concentrations of catecholamines have been noted also earlier in experimental anaphylactic shock in guinea pigs, the highest concentrations being in cases of protracted shock [14].

It appears from the present experiments that blood CA concentrations were high at death, which also supports the idea that they have a role as "antidote drugs." On the other hand, CAs may have a role in the mechanism of death via their known cardiotoxic effect.

Acute cardiac failure is thus also a possibility as the final event in anaphylactic death. Other compounds besides catecholamines that could cause myocar-

dial damage are PAF and, possibly, histamine. There is some evidence for an effect of PAF on the circulation [11], and the mechanism could be a direct toxic effect on myocardial cells. Histamine also seems to have an effect on the heart, causing tachycardia and heart block, which could be reduced by mepyramine and cimetidine [15].

The observation of high cortisol values at death is also a sign of countermeasures against the lethal shock in the anaphylaxis groups. Cortisol is a hormone that stabilizes the membrane of histamine-containing cells, e.g., mast cells and basophils, and thus can be regarded as one of the main tools involved in preventing the shock in anaphylactic reaction.

Thus, it might be possible to use its concentration in postmortem blood as a test for anaphylactic reaction.

The diagnosis and mechanism of anaphylactic death are still complicated and unclear, and studies on the role of less well-known mediators are necessary. It seems that two findings are most significant in anaphylactic death, namely high serum histamine values and acute emphysema resulting from bronchoconstriction. High catecholamine and cortisol values in the blood could be regarded as an additional sign, but this could reflect asphyxia following bronchoconstriction rather than being a primary phenomenon in anaphylaxis. The same argument could perhaps be applied to cortisol, but on the other hand there is some evidence that it may have a role in stabilizing the pathophysiological reactions occurring in the first phase of anaphylaxis. Postmortem assays of histamine, catecholamines and cortisol are lacking in connection with this type of death in humans, however.

Further, two kinds of difficulties remain when the results of the postmortem assays are interpreted. First, the concentrations of the hormones are likely to be changed after death as a result of diffusion from the endocrine glands and/or from the tissues into the blood or of bacteria which produce compounds in measurable amounts. For instance, the histamine content in the blood begins to be increased 24–48 h after death due to bacterial action [16].

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