EFFECT OF CATECHOLAMINES ON ERYTHROCYTE MECHANICAL FRAGILITY

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SUMMARY: Erythrocyte mechanical fragility in cells incubated with epinephrine and norepinephrine at different concentrations (10^{-9} to 10^{-5} M) was compared with the values obtained from the control suspensions which did not contain catecholamines. Norepinephrine had no significant effect on the mechanical fragility at any of the concentrations, while epinephrine caused a significant increase in erythrocyte mechanical fragility at 10^{-7} M concentration. At higher or lower concentrations of epinephrine there were no significant changes in erythrocyte mechanical fragility. The response was dose dependent. Epinephrine may therefore play a role in affecting blood flow in the microcirculation by changing the deformability of red blood cells, in addition to its known effects on the vessel walls. Further study is required to determine the dominant adrenergic receptor in this effect.

Key Words: Catecholamines, β-adrenergic, erythrocyte mechanical fragility.

INTRODUCTION

Catecholamines are released in response to stress in order to maintain homeostasis (5). Catecholamines also are used routinely in the treatment of cardiovascular diseases. Their effects on the vascular system are well established (6), but reports on their effects on the red blood cell are conflicting. It has however been shown that red blood cells are sensitive to vasoactive substances (1). The presence of Catecholamine receptors, especially of β adrenergic receptors on the red cell membrane has been demonstrated (3,11,12,13). Possible structural changes in the red blood cell brought about by the catecholamines affect the mechanical properties of erythrocytes, thus leading to changes in the blood flow in small vessels. In our study the effects of epinephrine (an α and β adrenergic agonist) and norepinephrine (an α adrenergic agonist) on the mechanical fragility of red blood cells, which indicates the changes in mechanical properties, were investigated by an *in vitro* method.

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MATERIALS AND METHODS

Blood samples

Heparinized venous blood was obtained from healthy male volunteers aged between 26 and 35 years. Erythrocytes were washed three times using an isotonic buffer (140 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 6 mM Glucose, 10 mM Tris, pH adjusted to 7.4), and buffy coats were carefully removed at each washing step. Washed erythrocytes were suspended with the same buffer to give a hematocrit of 10%. In each experiment, the prepared erythrocyte suspension was divided into 7 aliquots. The first one was used as the control suspension and contained no catecholamines. Epinephrine was added to the three samples so as to give final concentrations of 10^{-9} M, 10^{-7} M and 10^{-5} M. To another group three samples of nor-epinephrine were added to obtain the same final concentrations. All samples were incubated for 15 minutes on a mechanical shaker at 37°C. At the end of the incubation period, erythrocyte mechanical fragility was determined immediately.

Erythrocyte mechanical fragility measurements

Erythrocyte mechanical fragility was determined by using a technique modified from Nordt (8). Two ml of the incubated erythrocyte suspension were filtered through the 3 μm pore sited

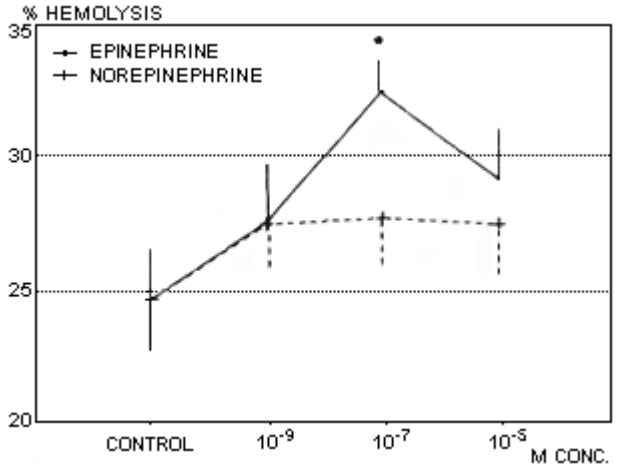

Figure 1: Effect of various concentrations of norepinephrine and epinephrine on mechanical fragility of erythrocytes (*:Difference from control value; p<0.05).

polycarbonate filters (Nuclepore, Lot:62B5D20) under 200 mm Hg pressure. The filtrate was centrifuged at 2500 rpm for 5 minutes and the hemoglobin concentration of the supernatant was determined by the cyanomethemoglobin method. The mechanical hemolysis ratio was calculated by dividing the hemoglobin concentration of the erythrocyte suspension before filtration. All specimens were studied in triplicate and their mean was used as the mechanical fragility of that specimen.

Statistical evaluation

Mechanical fragility values of the suspension containing different concentrations of epinephrine and nor epinephrine were compared with the control values separately using the Wilcoxon paired test.

RESULTS

Results are summarized in Figure 1. Mechanical hemolysis values were not changed significantly in the suspensions incubated with varying concentrations of norepinephrine. On the other hand, there is a significant increase in the mechanical fragility of the erythrocytes incubated in a medium containing 10^{-7} M epinephrine. In the suspensions containing 10^{-9} and 10^{-5} M epinephrine, the mechanical fragility ratios were not significantly different from the control values.

DISCUSSION

Erythrocytes are very special cells. They can change their shape extensively to pass through narrow channels i.e. the capillary blood vessels, which have smaller diameter than the red cell. This property of red cells is very important for proper function in the microcirculation. Any changes in these mechanical properties of red cells are expected to affect the tissue perfusion (4).

As a result of this unique property, normal red blood cells are capable of passing through the 3 μm pores used in our method, during which a certain degree of hemolysis takes place. Any change in the deformability of red blood cells result in an increased hemolysis ratio during the passage of erythrocyte suspensions under high pressure. The changes in this hemolysis ratio reflect the changes in mechanical fragility.

Many factors are known to affect the red cell mechanical properties. Among these are the Catecholamines released during stress (7). The nature of catecholamine effects on erythrocyte mechanical properties are not clearly understood. Pfafferot *et al.* (9) reported that norepinephrine caused a decrease in cell deformation at a concentration of 10^{-5} M, at low shear stresses. However, in our study, nerepinephrine seems to be ineffective at all concentrations, including the 10^{-5} M, but the stress range was not comparable with the study mentioned above. Pfafferot *et al.* (9) also reported that isoprenaline, a β adrenergic agonist, improved mechanical properties at lower concentrations (10^{-5} M and 10^{-7} M) and low shear stresses. Also Baar (2) demonstrated that while epinephrine had no effect on filtrability at 10^{-9} concentration, norepinephrine caused a decrease in this parameter at the same concentration.

In contrast to the above study, Allen *et al.* (1) demonsrated that at 10^{-9} M concentration epinephrine caused a maximum decrease in filtrability, indicating an impairment in the mechanical properties, in a dose dependent manner. Rasmussen *et al.* (10) reported that epinephrine was more effective than norepinephrine, and caused a decrease in filtrability and an increase in hypotonic hemolysis at 10^{-9} concentration. The results of our study support these findings with respect to epinephrine, indicating that this stress hormone may have a detrimental effect on the mechanical properties of the red blood cells and these changes may result in an impaired tissue perfusion.

According to our findings, catecholamine effects on the red blood cells may be mediated by the β adrenergic receptors on the membrane. Due to the conflicting results, further study is required.

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