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UREA FRAGMENTS DRUG TREATED HUMAN ERYTHROCYTES BY EXTERNALIZATION WITHOUT TEMPERATURE INCREASE

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ABSTRACT

Application of an appropriate amount of urea or urea with drug into a suspension of human erythrocytes in microcapillaries incubated with or without crenating or cup-forming drug in 5mM Hepes buffered saline, pH 7.4, at room temperature (25°C), resulted in the incorporation of urea into the membrane and induction of cell shape change of echinocytic type and fragmented the cells by externalization without causing haemolysis. The time to completion of urea induced cell fragmentation by externalization phenomenon was not influenced by drug concentration. It is suggested that urea was not an alternative to heat for the study of drug membrane interaction when cytoskekeleton are reduced. Based on these results, the effects of exposure of human erythrocytes to urea environment are discussed.

Keywords: urea- therapeutic use; fertilizer production environment, human erythrocytes,

INTRODUCTION

It has already been demonstrated that in heated human erythrocytes, in the absence or at drug concentration which are low compared with those required to cause significant shape change at room or physiological temperatures (Deuticke, 1968; Fujii et al. 1979, Glaser1979, 1982)), significant morphological changes occur at the thermal denaturation temperature of the structural protein, spectrin (Coakley et al 1983 Coakley and Deeley, 1980, Nwafor and Coakley 1985), the principal erythrocyte cyto skeleton, without causing haemolysis. When erythrocytes are heated through the thermal denaturation temperature of the structural protein spectrin they undergo one of two types of shape change either by developing a wavy out line (externalization) on the cell rim in less than a second and vesicles pinch from the wave crests in a time of the order of a second, or no wave grows on the cell rim but the cell membrane suddenly become cup- shaped and internalized at the dimple. The membrane stiffness can be scored by measuring the average number of waves per cell or the average number of internalized cells in a heated sample (Coakley and Deeley 1980. Nwafor and Coakley 1985). The rapid membrane invagination or evagination which occurred at the moment of spectrin thermal denaturation led to the suggestion that at physiological temperatures, spectrin acts against the expression, as shape changes, of the bending stresses associated with the membrane. Thus the thermal weakening of spectrin constraint showed that the bilayer shape is much more sensitive to ionic environment than studies of erythrocytes at 37°C suggests; and that erythrocyte shape change from which mechanical properties of membrane are deduced are really studies of the properties of spectrin and owe little to bilayer properties (Coakley and Deeley, 1980, Coakley and Gallez.1990). These studies led the present authors to examine if urea solution rather than heat could be used to denature intact human erythrocyte membrane cytoskeleton in the presence or absence of drug and the induction of the cell shape changes by perturbing the membrane structure which can be observed under the light microscope, from which our understanding of the structural properties of bilayer membranes and drug membrane interactions may emerge.

MATERIALS AND METHODS

Fresh human erythrocytes were obtained by finger pricking in 5mM Hepes buffered saline (145mM NaCl, pH 7.4), and washed three times and were finally resuspended in buffer (control) or in buffer containing a known concentration of cationic drug (tetracaine (<8.0mM), chlorpromazine (<0.2mM) or anionic drug (indiomethacin (<8.0mM), barbitone (<50mM)). The morphological changes of the human erythrocytes in hepes buffered saline without drug (control) or in buffered saline containing drug was studied following application of solution of urea with a drug or pure urea solution to cells in 5cm long rectangular cross- section micro capillaries of 0.1mm wall thickness, 0.1mm path length and 1.2mm width (Microslides, Camlab, Ltd)(fig.1). The morphological change was examined using Normarski differential interference optics and a television video analysis system.

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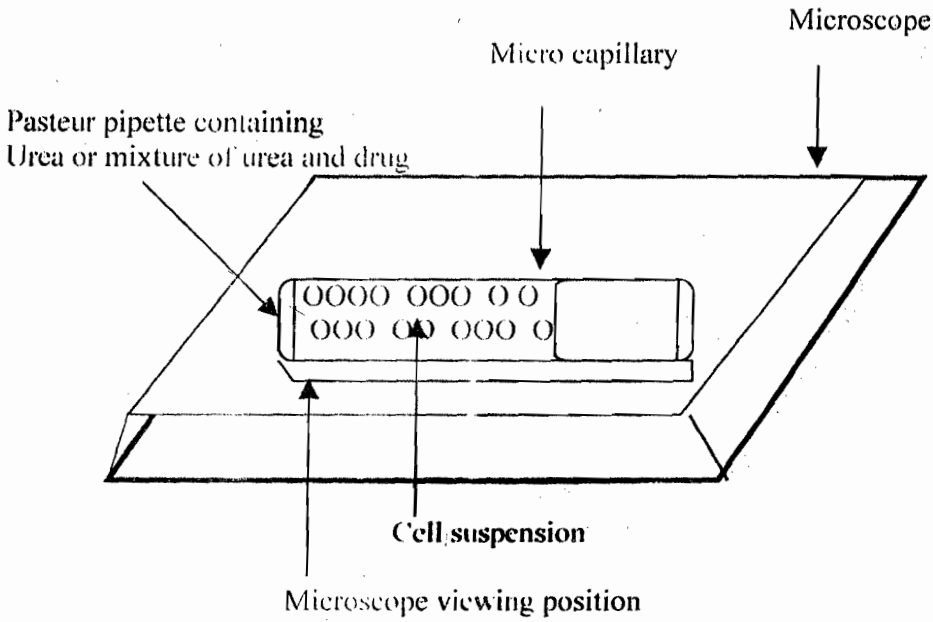

Fig. 1 A sketch of the system for the examination of vesicle production on human erythrocytes at constant temperature following exposure to solutions of urea or urea and drug.

Table 1. The influence of urea solution on the fragmentation pattern of human erythrocytes in different tetracaine concentrations. All the cells externalized membrane following urea denaturation of the membrane proteins.

Composition of urea Solution	Cell suspension medium of tetracaine (mM)	Time (mins) to membrane externalization completion
4 M urea	Control	15-18
"	0.5	15-18
"	4.0	15-18
"	8.0	15-18
6 M urea in 0.5 mM tetracaine	0.5	10-12
6 M urea in 4.0 mM tetracaine	4.0	"
6 M urea in 8.0 mM tetracaine	8.0	"
8 M urea in 5.0 mM tetracaine	0.5	2-5
8 M urea in 4.0 mM tetracaine	4.0	"
8 M urea in 8.0 mM tetracaine	8.0	"
10 M urea in 0.5 mM tetracaine	0.5	1-2
10 M urea in 4.0 mM tetracine		
10 M urea in 8.0 mM tetracaine	4.0	"
	8.0	"

RESULTS AND DISCUSSION

On addition of urea solutions to control cells (biconcave disc like shape) in microcapillaries, the erythrocytes gradually became echinocytic (best described as stage 1 and stage 2 membrane crenation (Fuji et al 1979, Glaser 1982)) and fragmented by externalization with vesicle formation on the spicules of the echinocytes without causing haemolysis. The time course of morphological change was studied: time zero was taken as that instant the first cell in the microscope field of 10 to 15 cells (fig 1) showed a slight movement on application of urea solution. The time to completion of

membrane fragmentation by externalization phenomenon was then measured from this zero point. Application of 4.0 M urea solution externalized membranes in times in excess of 15min (Table 1.) When cells treated with drug were placed in microcapillaries, the cells adopted the shape applicable to a particular drug (cup shape/ stomatocyte/ invagination/ internalization for cationic drugs; or externalization/ crenation/echinocytic/evagination for anionic drugs). When 4.0M urea solutions were added to drug treated cells in micro capillaries, urea changed the cup-shape induction of tetracaine or chlorpromazine to low incidence of echinocytic forms; and subsequently the erythrocytes fragmented by membrane externalization also without causing heamolysis. Increasing drug concentration at constant urea concentration had no effect on time to the completion of membrane externalization (Table 1). Table 1 shows also that when solution of urea and drug were added to cells in micro capillaries, the time to cell fragmentation by membrane extermination was not influenced by drug concentration. Increasing urea concentration in drug solution decreased the erythrocyte membrane fragmentation time, but for all drug and any concentration tested, the number of vehicles formed from the fragmenting cells were so large that they could not be resolved and counted. These are in contrast to the situation for erythrocytes heated in microcapillaries through the thermal denaturation temperature of the structural membrane protein spectrin at heating rate of 0.5°C/s were they change shape either by developing a wavy profile on cell rim and producing vesicles in a time of the order of a second, or the cells become stomatocytes and the membrane internalized at the cell dimple. (Nwafor and Coakley 1991, Coakley et al 1983). The results obtained when erythrocytes exposed to the other drugs tested were stressed with urea are generally consistent with those of Table 1. The results show that urea treated cells could not give quantitative measurements on the morphological responses or give the previously reported (Coakley et al 1983, Nwafor and Coakley 1995) effects of drugs on erythrocyte shape when the cytoskeleton are denatured. It is suggested that urea was not an alternative to heat for the study of drug induced human erythrocyte shape change when cytoskeleton are reduced and cautions the therapeutic use of urea or the effect of exposure of erythrocytes to urea production environment (like the fertilizer production environment) since urea can fragment erythrocytes by externalization without temperature increase in a time-- concentration dependent fashion.

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