EFFECT OF PULSED HYDRODYNAMIC INFLUENCES ON BLOOD CELLS IN VITRO AND IN VIVO

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The ever widening range of extremal ecologic influences acting on man in connection with the exploration of the hydrosphere, space flights, industrial catastrophes, and so on, necessitates an assessment of the resistance of the body and the study of the response of its functional and structural elements to a wide range of dynamic influences. It is in this connection that we have studied the response of blood cells in vitro and in vivo to pulsed hydrodynamic influences (PHDI), similar to percussive in character.

EXPERIMENTAL METHOD

Conserved human blood from donors and also heparinized blood freshly obtained from laboratory rats were used for the in vitro investigations. The blood was collected in elastic rubber bags and subjected to PHDI, once or five times (at intervals of 100-200 msec) with an excess pressure (ΔP) of 40 kPa and with $\tau^+ = 1.57$ msec. The control specimens were subjected to the same manipulations, but without exposure to PHDI. The experiments in vivo were carried out on male noninbred albino rats weighing 200-230 g and mice weighing 18-20 g. The animals were fixed by the limbs and head and exposed once or five times to PHDI with $\Delta P = 42.5$ kPa and $\tau^+ = 3.15$ msec (rats) or $\Delta P = 40$ kPa and $\tau^+ = 1.57$ msec (mice). The equally effective levels of PHDI chosen induced multiple petechial hemorrhages in the animals' lungs and caused barotrauma to the ears (the other organs showed no structural changes). Animals fixed but not exposed to PHDI served as the control. Blood for staining with acridine orange (AO, 20-100 μl) was taken from the retro-orbital sinus 15-30 min before and 30-60 min after exposure (for isolation of lymphocytes) after decapitation under superficial ether anesthesia. Isolation of leukocytes and lymphocytes, staining with AO, the lysosomal cationic test, and determination of Na^+, K^+, Mg^{2+}-dependent adenosine triphosphatase (ATPase) activity, adenylate cyclase (AC; EC 4.6.1.1) activity, and guanylate cyclase (GC, EC 4.6.1.2) activity in lymphocyte membrane preparations were carried out as described in [4, 6, 7]. The results were subjected to statistical analysis by Wilcoxon's V and paired T nonparametric tests [3].

EXPERIMENTAL RESULTS

Exposure to PHDI led to increased AO accumulation by human, rat, and mouse blood cells both in vitro (Fig. 1a) and in vivo (Fig. 1b). According to the results of the investigations in vitro, the response of whole blood was virtually indistinguishable from that of purified leukocytes or lymphocytes; erythrocytes, however, did not react to PHDI according to this criterion (Fig. 1a). Thus changes in the staining properties of the blood in response to exposure were due mainly to the reaction of the white blood cells. The results in Fig. 1 also indicate that blood cells reacted differently to one and five exposures to PHDI: the effect of multiple exposures in vitro and in vivo was appreciably weaker than that of a single exposure.

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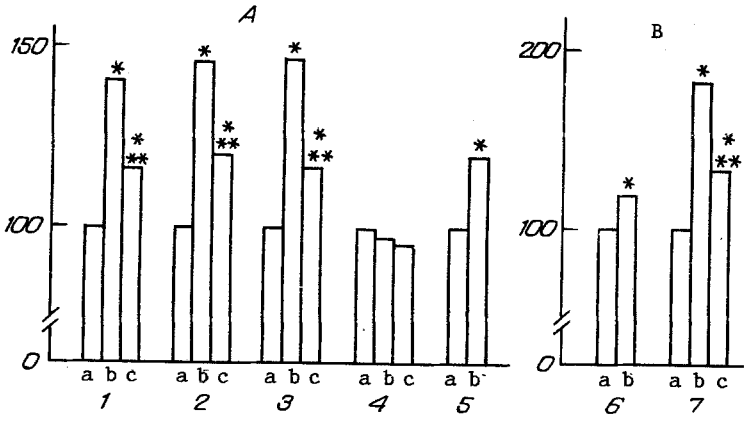

Fig. 1

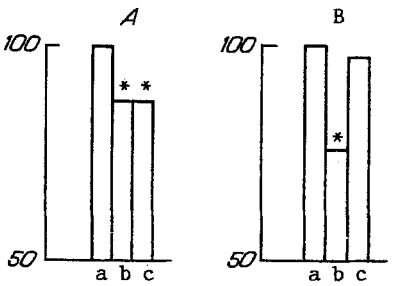

Fig. 2

Fig. 1. Effect of pulsed hydrodynamic influences (PHDI) on accumulation of AO by human and laboratory animal blood cells in vitro (A) and in vivo (B). Vertical axis, quantity of AO bound with cells (in per cent of control). a) Control, b) single exposure, c) five exposures. 1) Whole human blood, 2) human leukocytes, 3) human lymphocytes, 4) human erythrocytes, 5, 7) whole rat blood, 6) whole mouse blood. 1, 4) 2-4 experiments in each case, $n = 4$; 5-7) 1 experiment in each case, $n = 8$. $^{*}p < 0.05$ indicates significant difference compared with *a*, $^{**}p < 0.05$ significant difference compared with *b*.

Fig. 2. Effect of PHDI on secretory degranulation of neutrophils in vitro (A) and in vivo (B). Vertical axis, mean cytochemical coefficient (in per cent of control); a) Human blood, 3 experiments, $n = 4$, 100% — 1.05 + 0.016, b) Rat blood, 2 experiments, $n = 6$, 100% — 1.31 + 0.30.

It follows from the data in Fig. 2a that exposure in vitro led to a definite decrease in the content of cationic proteins in the neutrophilic granulocytes (neutrophils). No appreciable differences were noted between a single exposure and five exposures. A single exposure to PHDI in vivo also led to a decrease in the content of cationic proteins in the neutrophils, whereas after five exposures no changes were found in this parameter (Fig. 2b). These results are evidence of stimulation of secretory degranulation of the neutrophils by PHDI.

Exposure to PHDI led to a decrease in ATPase activity both in vitro and in vivo. Five exposures in vivo had a weaker effect on activity of the enzyme than a single exposure. Changes in AC activity under the influence of PHDI were different in character: exposure led to a definite increase in activity of this enzyme in vitro and a decrease in vivo. No appreciable differences could be found between the action of 1 and 5 exposures to PHDI. Changes in GC activity were not found as a result of exposure to PHDI. Thus the results of the experiments in vitro point to a direct response of the white blood cells to PHDI. The similar trend of the changes discovered in vitro and in vivo suggest the possibility of a direct influence of mechanical disturbances of the biological environment caused by PHDI on the blood cells in the blood stream during exposure. It was noted that five exposures regularly gave a weaker effect than a single exposure, whether adsorption of AO or secretory degranulation of neutrophils was used as the test; similar results also were obtained in a study of enzyme activity. These bindings suggested that repeated PHDI under certain conditions can lead to a lowering of reactivity (a kind of habituation) of the white blood cells to subsequent influences of the same intensity.

An increase in AO accumulation, which is considered to be a classical sign of excitation or injury of a cell [1, 4], evidently indicates increased permeability of the cell membrane. Degranulation of the neutrophils under physiological conditions takes place on contact of the cell membrane of the neutrophils with several biologically active substances of microbial origin [5]. The cell responses to PHDI observed, namely degranulation of neutrophils, transmembrane diffusion of AO, changes in enzyme activity of integral membrane proteins — are considered to be based on common mechanisms controlling the properties of the cell membrane. Under these circumstances repeated exposures to PHDI may induce relative stabilization of the cell membranes, possibly through the organization of its liquid-crystal lipid structure, or possibly by influencing transmembrane transport of ions and covalent modification of membrane proteins.

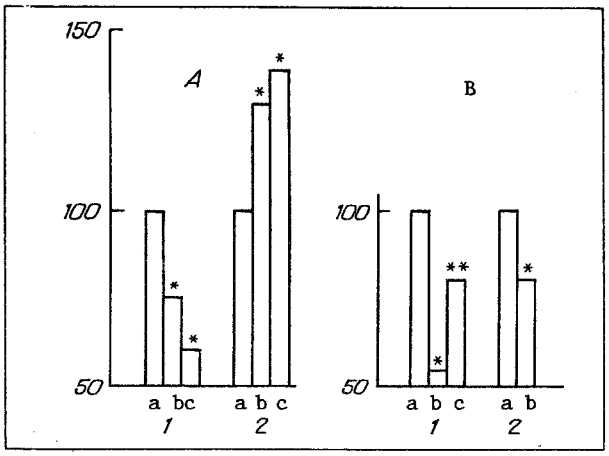

Fig. 3. Effect of PHDI on Na^+,K^+,Mg^{2+}-dependent ATPase (1) and on AC activity (2) of lymphocyte membranes in vitro (A) and in vivo (B). Vertical axis, activity (in per cent of control). a) Human blood, b) rat blood. A, 1) 4 experiments, n = 4, 100% — 39 nmoles/mg protein·min, A, 2) 9 experiments, n = 4, 100% — 50.5 pmoles/mg·min, B, 2) 1 experiment, n = 8, 100% — 48.5 pmoles/mg·min. Remainder of legend the same as to Fig. 1.

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