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The influence of prostaglandins on pig, cattle and horse erythrocytes during hypotonic NaCl – induced haemolysis

Wpływ prostaglandyn na erytrocyty świń, bydła i koni w czasie hemolizy indukowanej hipotonicznymi roztworami NaCl

One of essential physiological properties of prostaglandins is their cytoprotective activity on various cells, consisting in their protection against the influences of noxious agents. Currently the investigations are focused mainly on their cytoprotective action in the gastrointestinal tract. So far, very little attention has been paid to such activity on red blood cells.

Cytoprotective activity of prostaglandis on red blood cells was stated by several authors (1, 5, 6, 7, 10), yet, the mechanism is still insufficiently recognized. There is no doubt that this process starts by a conjunction of the prostanoid with the adequate receptor on the outer surface of the cell membrane (2, 14). The nature of this receptor, however, is still little known (4, 9).

In our earlier papers (5, 6) we have presented thy cytoprotective effect of prostaglandin E_1 and E_2 on pig erythrocytes.

In the present paper we will present the influence of prostaglandins PGE_1, PGE_2, $PGF_1\alpha$ and $PGF_{2\alpha}$ on pig, cow and horse erythrocytes during hypotonic NaCl – induced haemolysis. Since various authors have observed different intensity of the cytoprotective effect with respect to a kind of the haemolytic agent (3, 5, 11), we shall also establish whether this effect depends on the power of acting agent (NaCl concentration).

MATERIAL AND METHODS

The blood of pigs (Great White Polish) and cows (Lowland Black White) was taken in a local slaughter-house and the blood of horse (Little Polish with an admixture of cold blood) was taken from clinically healthy animals in the Clinic of Surgery of our Veterinary Faculty, Agricultural Academy, Lublin. In all cases, heparin was used as an anticoagulant. Blood was centrifuged for 15 min at 200 g, plasma was removed together with the buffy coat, and the erythrocytes were washed three times

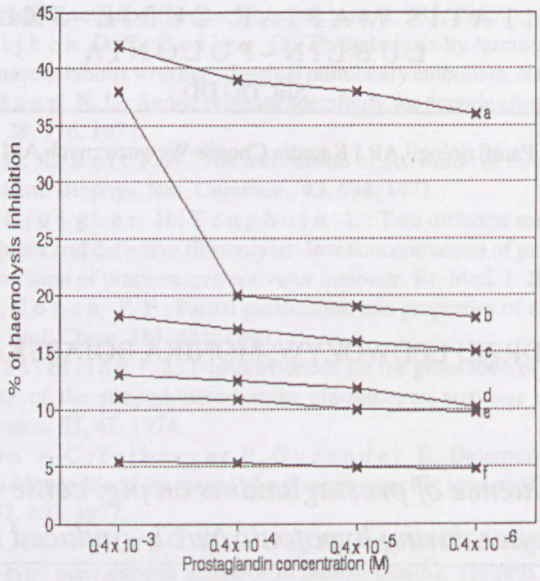

Fig. 1. The per cent inhibition of pig erythrocyte haemolysis by prostaglandin PGE_1 and PGE_2 induced by various NaCl concentrations; a – PGE_1, 0.094 M NaCl, b – PGE_2, 0.094 M NaCl, c – PGE_1, 0.086 M NaCl, d – PGE_2, 0.86 M NaCl, e – PGE_1, 0.077 M NaCl, f – PGE_2, 0,077 M NaCl

with the cold (277°K) 0.154 MNaCl solution. Then, a suspension of red blood cells (1:20) was made in 0.15 M phosphate buffer, pH = 7.4. Prostaglandins PGE_1, PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ were kindly provided by Prof. D. H. Nugteren, Unilever Res. Lab., Vlaardingen, Netherlands. The stock solution of prostaglanins (2.6×10^{-2} M) was made in absolute ethanol. The working solutions (2.6×10^{-3} M; 2.6×10^{-4} M; 2.6×10^{-5} M; 2.6×10^{-6} M) were prepared using the above buffer.

In order to establish the osmotic resistance of erythrocytes, a series of NaCl solutions with concentrations decreasing from 0.120 M to 0.043 M in 0.09 M intervals were made. The mixture consisting of 4.5 ml NaCl solution and 0.5 ml of the erythocyte suspension was incubated for 30 min. at room temperature. After incubation, the samples were centrifuged for 10 min. at 2,000 g and the optical density of the supernatant was measured at 540 nm. The optical density of the supernatant of the sample in which haemolysis was induced with distilled water was assumed as 100% haemolysis.

One half ml of the erythrocyte suspension was given to 0.1 ml of the working solutions of the prostaglandins (obtaining final concentrations of 0.4×10^{-4} M; 0.4×10^{-5} M; 0.4×10^{-6} M) and the mixture was incubated for 30 min at the room temperature. Next, the samples containing pig erythrocytes were given to 4.4 ml of 0.094 M, 0.086 M or 0.077 M NaCl solution, the samples of cow erythrocytes were mixed with 4.4 ml of 0.086 M, 0.077 M or 0.068 M NaCl, the samples of horse erythrocytes were added to 4.4 ml of 0.103 M; 0.094 M or 0.086 M NaCl.

The samples were incubated again for 30 min in the room temperature, centrifuged for 10 min at 2,000 g and the optical density of the supernatant was measured at 540 nm. The control sample were treated analogously, by adding 0.1 ml of buffer instead of the prostaglandin solution. Each trial was performed in size replications.

Percentage of the hemolysis inhibition for each of the NaCl concentrations was calculated by a comparison of the optical density of the supernatant of sample incubated with prostaglandin with that incubated with water, which was assumed as 100% of haemolysis.

RESULTS

Figure 1 presents the cytoprotective effect of the prostaglandins on the pig erythorocytes during various NaCl concentrations–induced haemolysis. Both PGE_1 and PGE_2 in concentration of 0.4×10^{-3} produce a comparable effect. With concentrations of 0.4×10^{-4} M to 0.4×10^{-6} M the effect of PGE_1 is about twice stronger than the effect of PGE_2.

Acting on the horse erthrocytes, all the examined prostaglandins showed a similar activity (Figs 2 and 3). In this case PGE_1 showed the strongest cytoprotective activity, while $PGF_{2\alpha}$ showed the slightest one.

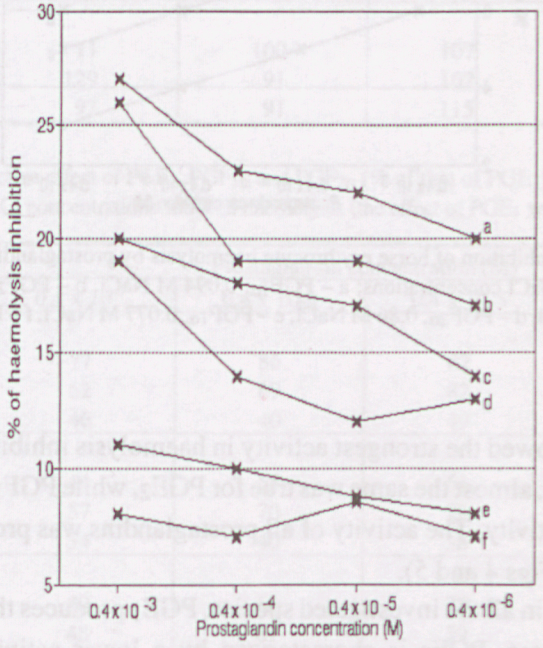

Fig. 2. The per cent inhibition of horse erythrocyte haemolysis by prostaglandin PGE_1 and PGE_2 induced by various NaCl concentrations; a – PGE_1, 0.094 M NaCl, b – PGE_2, 0.094 M NaCl, c – PGE_1, 0.086 M NaCl, d – PGE_2, 0.86 M NaCl, e – PGE_1, 0.077 M NaCl, f – PGE_2, 0.077 M NaCl

Tab. 1. The cytoprotective effect PGE_2 (% of that of PGE_1) on pig erythrocytes during various NaCl concentrations-induced haemolysis (the effect of PGE_1 was assumed as 100%)

NaCl	Prostaglandin concentration (M)			
	0.4×10^{-3}	0.4×10^{-4}	0.4 x 10-5	0.4 x10-6
0.094 M	56	51	51	49
0.086 M	74	75	73	86
0.077 M	59	55	54	52

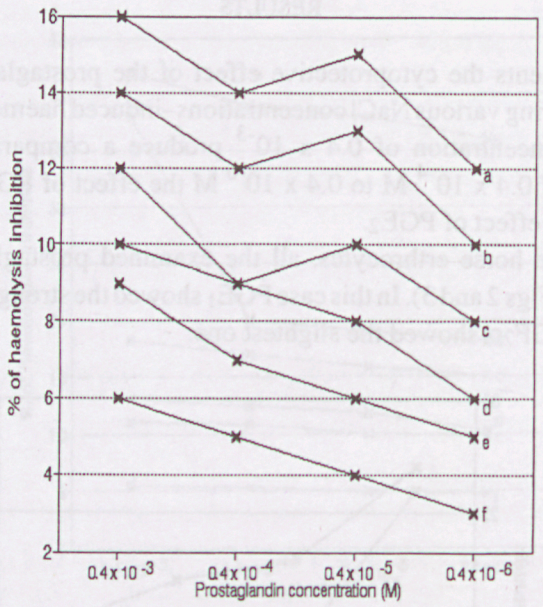

Fig. 3. The per cent inhibition of horse erythrocyte haemolysis by prostaglandins $PGF_{1\alpha}$ and $PGF_{2\alpha}$ induced by various NaCl concentrations; a – $PGF_{1\alpha}$, 0.094 M NaCl, b – $PGF_{2\alpha}$, 0.094 M NaCl, c – $PGF_{1\alpha}$, 0.086 M NaCl, d – $PGF_{2\alpha}$, 0.86 M NaCl, e – $PGF_{1\alpha}$, 0.077 M NaCl, f – $PGF_{2\alpha}$, 0,077 M NaCl

PGE_1 also showed the strongest activity in haemolysis inhibition in the case of cow erythrocytes; almost the same was true for PGE_2, while $PGF_{1\alpha}$ and $PGF_{2\alpha}$ had a little weaker activity. The activity of all prostaglandins was proportional to their concentrations (Figs 4 and 5).

In most cases in all the investigated species, PGE_1 produces the strongest effect on the erythrocytes. PGE_2 is characterized by a lower activity when used in concentrations of 0.4×10^{-4}, 0.4×10^{-5} M and 0.4×10^{-6} M in the case of pig erythrocytes during 0.094 M and 0.077 M NaCl–induced haemolysis (about 50% of the PGE_1 effect). This activity is stronger in the case of 0.086 M NaCl–induced haemolysis (75% of the PGE_1 effect, Tab. 1).

Acting on cow erythrocytes during 0.077 M NaCl–induced haemolysis, PGE_2 has the activity power equal to PGE_1 and in the case of 0.068 M NaCl–induced haemolysis it surpasses the power of this prostaglandin slightly. $PGF_{1\alpha}$ and $PGF_{2\alpha}$ show comparable, though lower activity (Tab. 2).

The haemolysis of horse erythrocytes induced by 0.094 M NaCl and 0.077 M NaCl is inhibited in an equal degree by PGE_2 and $PGF_{1\alpha}$ while $PGF_{2\alpha}$ shows much lower activity (Tab. 3).

Tab. 2. The cytoprotective effect of PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ (% of that of PGE_1) on cow erythrocytes during various NaCl concentrations-induced haemolysis (the effect of PGE_1 was assumed as 100%)

	Prostaglandin concentration (M)			
	0.4×10^{-3}	0.4 x 10-4	0.4 x 10-5	0.4 x 10-6
0.086 M NaCl				
PGE_2	59	59	94	90
$PGF_{1\alpha}$	47	45	60	56
$PGF_{2\alpha}$	51	48	62	54
0.077 M NaCl				
PGE_2	93	79	84	90
$PGF_{1\alpha}$	72	65	68	57
$PGF_{2\alpha}$	73	68	63	51
0.068 M NaCl				
PGE_2	11	100	107	100
$PGF_{1\alpha}$	129	91	107	92
$PGF_{2\alpha}$	92	91	115	79

Tab. 3. The cytoprotective effect of PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ (% of that of PGE_1) on horse erythrocytes during various NaCl concentrations-induced haemolysis (the effect of PGE_1 was assumed as 100%)

	Prostaglandin concentration (M)			
	0.4×10^{-3}	0.4 x 10-4	0.4 x 10-5	0.4 x 10-6
0.086 M NaCl				
PGE_2	77	86	82	67
$PGF_{1\alpha}$	62	69	82	71
$PGF_{2\alpha}$	46	40	49	43
0.077 M NaCl				
PGE_2	44	55	51	48
$PGF_{1\alpha}$	57	70	79	67
$PGF_{2\alpha}$	34	40	38	36
0.068 M NaCl				
PGE_2	40	50	78	44
$PGF_{1\alpha}$	48	61	59	46
$PGF_{2\alpha}$	30	30	42	35

DISCUSSION

It is clear from the above data that PGE_1, PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ differ in degree of inhibition of the erythrocyte haemolysis of the species investigated. Prostaglandins of the F series have no effect on pig erythrocytes, which in agreement with our earlier observations (5). This fact can be explained by the lack of specific receptors on the red blood cell surface in this species, responsible for $PGF_{1\alpha}$ and $PGF_{2\alpha}$ binding, as both prostaglandins inhibit the haemolysis of horse and cow erythrocytes. For that reason, the lack of activity of both prostaglandins

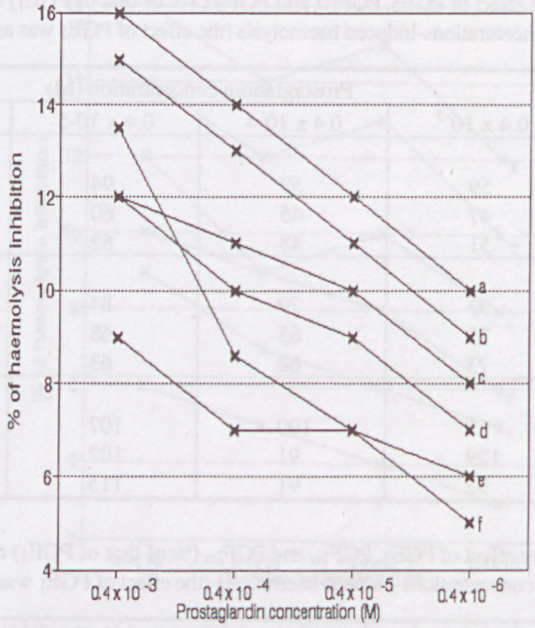

Fig. 4. The per cent inhibition of cow erythrocyte haemolysis by prostaglandins PGE_1 and PGE_2 induced by various NaCl concentrations; a – PGE_1, 0.086 M NaCl, b – PGE_2, 0.086 M NaCl, c – PGE_1, 0.077 M NaCl, d – PGE_2, 0.77 M NaCl, e – PGE_1, 0.068 M NaCl, f – PGE_2, 0,068 M NaCl

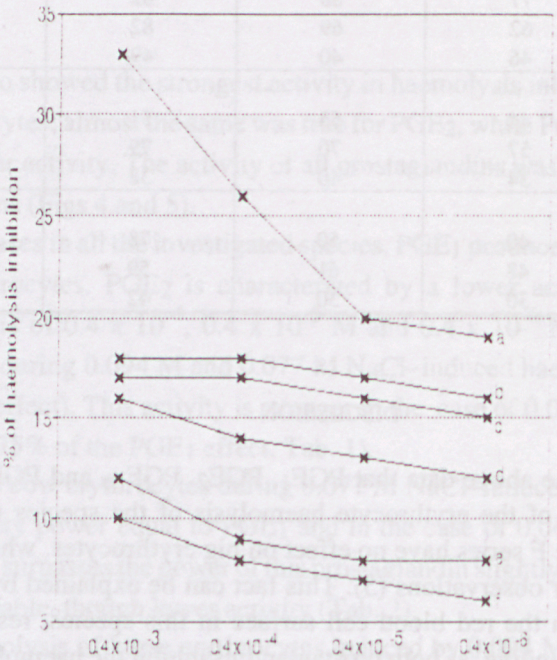

Fig. 5. The per cent inhibition of cow erythrocyte haemolysis by prostaglandins $PGF_1\alpha$ and $PGF_2\alpha$ induced by various NaCl concentrations; a – $PGF_1\alpha$, 0.086 M NaCl, b – $PGF_2\alpha$, 0.086 M NaCl, c – $PGF_1\alpha$, 0.077 M NaCl, d – $PGF_2\alpha$, 0.77 M NaCl, e – $PGF_1\alpha$, 0.068 M NaCl, f – $PGF_2\alpha$, 0,068 M NaCl

cannot be explained by the difference in chemical structure between prostaglandins of the E and F series. Such a difference can be the reason for the lack of cytoprotective effect of 16, 16-dimethyl-PGE_1 observed by Guth et al (3). Steric hindrance at C-16 of this analogue can be the reason for the lack of effect on erythrocytes exposed to aspirin or ethanol.

The cytoprotective effect depends largely on the power of a haemolytic agent (NaCl concentration). The main tendency is a decrease of this effect with an increase of hypotonicity of the NaCl solutions. At present, there is no standard method of red blood cell haemolysis during the investigation of cytoprotective effect of prostaglandins. As a haemolyzing agent, the authors used hypotonic NaCl solutions (6, 7, 9), aspirin (3, 5) ethanol (3) or a superoxide radical (11). It is known that susceptibility to haemolysis depends on many factors: the surface/capacity ratio of the red blood cell which changes significantly with respect to a species, the differences in the elasticity of the cell membrane and the cell turgor (12).

Taniguchi et al. (11) suggest that the resistance of erythrocytes to haemolysis and their susceptibility to the action of prostaglandins depends on the composition of phospholipids of the cell membrane. In spite of the fact that the phospholipid composition may play a certain role here, it does not seem to be a decisive factor. Wessels and Veerkamp (13) examining the erythrocytes of eight species of mammals did not state a correlation between the phospholipid composition of the cell membrane and its permeability for glycerol. The argument concerning the lack of correlation between the phospholipid composition of the membrane and its susceptibility to the action of prostaglandins can be confirmed by the statement of the cytoprotective effect of the prostaglandins of E and F series on atherosclerotic patients erythrocytes (7). This effect was the same as the one exerted on red blood cells of healthy individuals, despite significant differences in the phospholipid composition of the membrane between both groups.

The mechanism of the prostaglandin action on the cell membrane, however, is still unknown. Kury et al. (4) have shown that the action of PGE_1 and PGE_2 on erythrocytes changes the elasticity of acyl chains in their cell membrane and the susceptibility to deformation. This effect occurred only in the presence of Ca^{2+} ions in the incubation medium. If the calcium ions played any role in the origin of the effect (e.g. activating adenyl cyclase) the cytoprotective effect would be observed only in their presence. In this case, however, and earlier papers (5, 6) its occurrence would not be connected with the presence of extracellular calcium. The protective effect was also observed by Rasmussen et al. (9) during the incubation of erythrocytes with PGE_1 and PGE_2 in Ca^{2+} – free medium.

Recently Kobusiewicz et al. (8) have shown a strong cytoprotective effect of prostacyclin and its synthetic analogue Iloprost on ischemic myocardium in dogs. It seems that this effect is rather general and occurs in many cells of different origin.

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STRESZCZENIE

Badano wpływ prostaglandyn na erytrocyty zwierząt domowych w czasie hemolizy indukowanej hipotonicznymi roztworami NaCl. Erytrocyty świń, krów i koni różnią się między sobą opornością osmotyczną. Prostaglandyny PGE_1, PGE_2, $PGF_{1\alpha}$ i $PGF_{2\alpha}$ wywierają cytoprotekcyjny wpływ na erytrocyty badanych gatunków hamując hemolizę wywołaną hipotonicznymi roztworami NaCl. Prostaglandyna PGE_1 wywiera najsilniejsze działanie cytoprotekcyjne w stosunku do erytrocytów wszystkich badanych gatunków.