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Short communication

Foetal erythrocytes exhibit an increased ability to scavenge for nitric oxide

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Abstract

The presence of adult human whole blood inhibited in vitro relaxations of rat aortic rings by the nitric oxide (NO) donor *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP). Incubation with foetal blood containing the same concentration of haemoglobin produced a shift to the right of the relaxation curve. SNAP-induced vasorelaxations were more inhibited by dialysed solutions of haemoglobin than by the presence of erythrocytes in the organ bath, but there were no differences between the effect of adult or foetal haemoglobins. The presence of plasma from adult or foetal blood did not modify the effects of SNAP. Relaxations induced by endogenous, endothelium-derived, NO were more inhibited by foetal than by adult erythrocytes. These results suggest that foetal erythrocytes have a higher NO scavenging effect than those present in adult blood. © 1998 Elsevier Science B.V.

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1. Introduction

The synthesis of nitric oxide (NO) by vascular endothelium is essential for the vasodilator tone involved in the regulation of blood pressure (Moncada and Higgs, 1992). Although reversible interactions of NO with thiol groups in albumin (Stamler et al., 1992) and haemoglobin (Hb; Jia et al., 1996) have been described, the short half-life of free NO in blood is mainly a consequence of its rapid binding to Fe active sites located in the haem group of erythrocyte Hb (Wennmalm et al., 1992). These Fe sites are also responsible for the reversible combination of Hb with oxygen molecules (Delivoria-Papadopoulos and DiGiacomo, 1992). Erythrocytes in newly born infants contain predominantly foetal Hb (HbF), which shows a greater oxygen affinity than that of haemoglobin A (HbA) which is present in adults (Delivoria-Papadopoulos and DiGiacomo, 1992). If foetal erythrocytes would also display an increased affinity for NO, this would compete with the soluble guanylyl cyclase in the vascular smooth muscle, so that the amount of this mediator available for acting on the vessel wall would be decreased, and a reduction in vasorelaxing responses could be expected. Interestingly, newborns show important disorders related to an increased vasoconstrictor tone such as neonatal pulmonary hypertension (Kliegman, 1996) and necrotizing enterocolitis (Nowicki and Nankervis, 1994).

2. Materials and methods

Rat aortic rings were mounted in organ baths (5 ml) for isometric force recording under 2 g resting tension in Krebs's solution (37°C, pH 7.4, 95% O_2–5% CO_2). After a 60-min equilibration period, the presence of endothelium was evaluated by the addition of acetylcholine (10^{-6} and 10^{-5} M) in noradrenaline (10^{-6} M) precontracted rings, and only those exhibiting at least a 75% relaxation were used. Forty minutes later, tissues were contracted again with noradrenaline (10^{-6} M) and a cumulative concentration–response curve with the NO-donor, *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP, 10^{-9}–10^{-4} M, Wellcome Research Laboratories) was performed in the presence of human whole blood giving an Hb concentration of 60 μM in the bath medium. This concentration of Hb was selected from preliminary dose–response studies and is below that

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of normal blood (2.2–2.5 mM) (Guyton, 1991). Blood was obtained either from healthy adults or from umbilical cords collected after spontaneous births at the Department of Obstetrics of Hospital Clínico Universitario of Valencia. Control experiments were performed using equivalent volumes (100 μl) of saline, or plasma from adult or foetal blood from which erythrocytes had been removed by centrifugation. In some cases, samples from either source were centrifuged (3500 rpm), and the erythrocytes washed 3 or 4 times with saline before being hemolyzed for 2 h with an equal volume of distilled water and 0.4 ml of carbon tetrachloride. The hemolyzed mixture was centrifuged again, the Hb layer removed, and the process repeated until a clear solution was obtained. Thereafter, the two Hb solutions were placed in dialysis sacks at 4°C for 16 h with constant stirring of the outside solution (distilled water). SNAP-induced vasodilatations were performed in the presence of these dialysed samples giving an Hb concentration of 60 μM. In some cases, vessels were incubated with D-2,3-diphosphoglycerate (DPG, 80 μM).

In a second group of experiments, isolated rings were precontracted with noradrenaline (10^{-6} M) and cumulative concentration–response curves to acetylcholine (10^{-9}–10^{-5} M) were performed before and after the addition of foetal or adult erythrocytes yielding a 3 μM concentration of Hb in the organ bath. In control experiments, repeated exposures to acetylcholine elicited similar vasorelaxing responses.

The content of HbF in foetal blood was determined spectrophotometrically after alkaline denaturation of HbA (Brinkman and Jonxis, 1935). To exclude the possibility of our experimental conditions facilitating the lysis of erythrocytes, at the end of each experiment, the concentration of free Hb in the organ bath fluid was measured spectrophotometrically after centrifugation, and in all occasions was below the level of detection (10^{-7} M). The composition of the Krebs solution was (in mM) Na^+ 142, K^+ 6, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 127.8, HCO_3^- 24, $H_2PO_4^-$ 1.2, SO_4^{2-} 1.2 and glucose 11. All data are expressed as mean ± S.E.M. pD_2 value represents $-\log EC_{50}$, where EC_{50} is calculated in each individual experiment as the concentration of acetylcholine or SNAP necessary to induce an effect equal to 50% of the maximum relaxant effect. Comparisons between groups were performed by ANOVA followed by the Tukey test or by Student's *t*-test for paired or unpaired data, and a probability of $P < 0.05$ or less was considered as statistically significant. Unless stated otherwise, all drugs were obtained from Sigma.

3. Results

Addition of SNAP to the organ bath produced a concentration-dependent relaxation of the aortic rings (pD_2 6.32 ± 0.18, $n = 13$). Incubation with plasma from adult or foetal blood did not influence the vasorelaxant effects of SNAP, pD_2 6.28 ± 0.13 ($n = 23$) and 5.97 ± 0.09 ($n = 10$), respectively. The presence of adult whole blood in the medium produced a significant ($P < 0.01$) shift to the right of the relaxation curve induced by SNAP (pD_2 5.54 ± 0.11, $n = 13$). SNAP-induced vasorelaxations were significantly ($P < 0.05$) more inhibited in the presence of foetal blood (pD_2 5.05 ± 0.08, $n = 12$) containing the same concentration of Hb (60 μM) (Fig. 1A). The content of HbF in the foetal blood employed in our experiments represented 68 ± 3% of the total Hb amount. When dialysed Hb was present in the organ bath, it caused significantly ($p < 0.05$) greater inhibition of SNAP-induced vasodilatations than that induced by foetal or adult red cells containing a similar concentration of Hb (60 μM) (Fig. 1B). However, there were no differences between the actions of adult and foetal dialysed Hb, with pD_2 values of 4.73 ± 0.06 ($n = 7$) and 4.62 ± 0.17 ($n = 5$), respectively. Addition of DPG (80 μM) to the medium did not influence the effects of dialysed adult (pD_2 4.63 ± 0.13, $n = 5$) and foetal (pD_2 4.55 ± 0.22, $n = 4$) Hb.

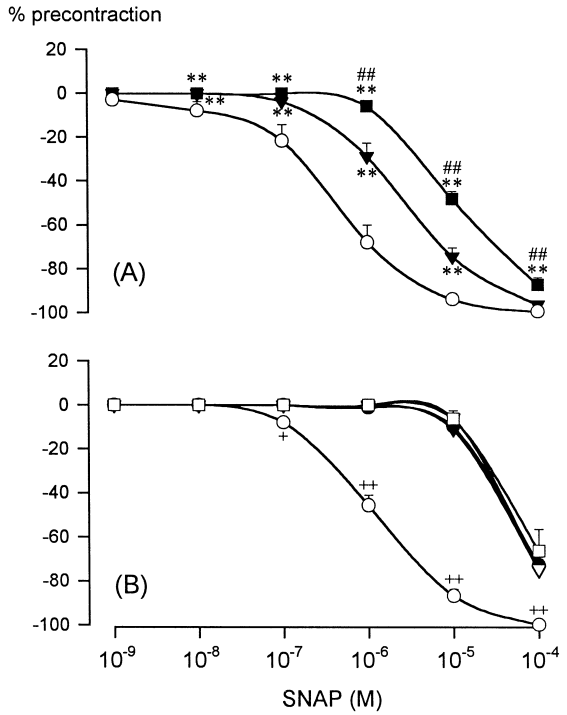

Fig. 1. Relaxation of rat isolated aortic rings by SNAP. (A) Experiments were carried out in control conditions (○) or with adult (▼) or foetal (■) blood in the organ bath giving a haemoglobin concentration of 60 μM. (B) Relaxation by SNAP in control conditions (○) and in the presence of dialysed haemoglobin (60 μM) from adult (▽) or foetal (□) blood with (open symbols) and without (filled symbols) D-2,3-diphosphoglycerate (DPG, 80 μM). Results are expressed as mean ± S.E.M. of 4–23 experiments. $^{**}P < 0.01$ vs. control, $^{\#\#}P < 0.01$ vs. adult blood. $^{+}P < 0.05$ and $^{++}P < 0.01$ vs. adult and foetal dialysed haemoglobin with and without DPG (ANOVA + Tukey test).

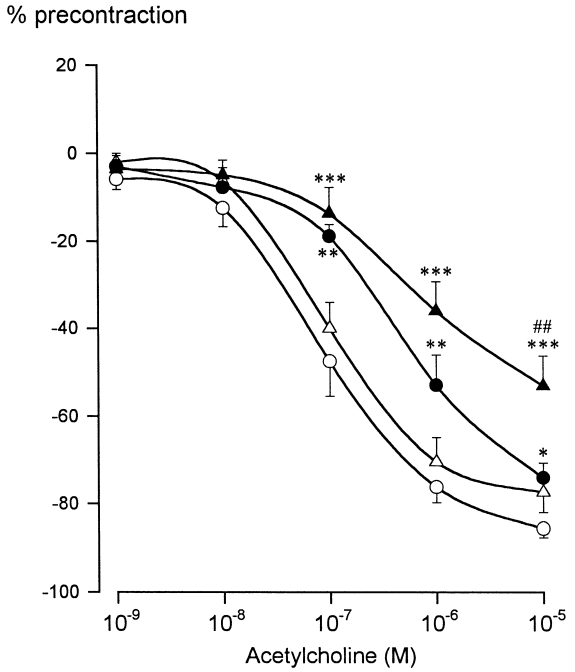

Fig. 2. Relaxations of rat isolated aortic rings induced by acetylcholine in the absence (open symbols) or presence of adult (▼) or foetal (■) blood yielding an haemoglobin concentration of 3 μM. Results are expressed as mean ± S.E.M. of 8 experiments. $**P < 0.01$ and $***P < 0.001$ vs. respective control (paired *t*-test) and $^{\#\#}P < 0.01$ vs. adult blood (unpaired *t*-test).

As shown in Fig. 2, the presence of adult erythrocytes induced a significant ($P < 0.05$) shift to the right of acetylcholine-induced, endothelium-dependent, relaxations (pD_2 6.40 ± 0.12 vs. 7.05 ± 0.17 respectively, $n = 8$,). However, this vascular response was more inhibited by foetal blood erythrocytes yielding a similar concentration of Hb (3 μM) with a significant change in pD_2 values (6.21 ± 0.29 vs. 6.97 ± 0.11, $n = 8$; $P < 0.05$) and a reduction in the maximum effect that differs significantly ($P < 0.01$) from that obtained with adult red cells.

4. Discussion

In the present study, the presence of adult whole blood in the medium produced a significant shift to the right of the relaxation curve induced by the nitric oxide donor, SNAP. Similar observations have been previously explained by the binding of NO by erythrocytes (Gillespie and Sheng, 1988), which reduces the amount of NO available for acting on the isolated vessel. Interestingly, SNAP-induced vasorelaxations were significantly more inhibited in the presence of foetal blood containing the same concentration of Hb. Incubation with plasma from either adult or foetal blood did not modify the effects of SNAP, thus suggesting that the presence of red cells is responsible for the diminution of the relaxation induced by the NO donor. Furthermore, the possibility that differences in the level of haemolysis between the two types of erythrocytes could result in changes in the interaction of Hb with NO have also been excluded, since no significant level of haemolysis was detected in our experimental conditions. Thus, the distinct behaviour between adult and foetal blood could be explained on the basis that erythrocytes containing HbF have a higher NO affinity than those containing HbA. However, the difference between HbA and HbF was not present when dialysed solutions were used. This implies that the distinct affinity for NO does not result from structural differences of the Hb molecules, but is a consequence either of interactions with other components within the erythrocyte or anatomical peculiarities between erythrocytes, which modulate the ability of NO to interact with Hb. In the classical studies evaluating oxygen affinity, the differences between HbA and HbF were also only present when intact erythrocytes were employed, and have been attributed to a greater inhibitory effect of intracellular DPG on HbA (Delivoria-Papadopoulos and DiGiacomo, 1992). In our experiments, however, the affinity for NO was not influenced when DPG was added to the organ bath at concentrations capable of modifying the oxygen affinity of dialysed solutions of HbA (Benesch and Benesch, 1969), thus suggesting that DPG is not involved in the differential NO affinity of adult and foetal erythrocytes.

The observation that endogenous, acetylcholine-stimulated, NO delivered from endothelial cells is scavenged more efficiently by foetal than by adult erythrocytes suggests that such a difference could be of physiological relevance. However, it needs to be emphasised that the difference in NO affinity observed in the present study would be increased in vivo, since we incubated the tissue with a relatively low number of red cells that yield Hb concentrations substantially smaller than those present in normal blood (Guyton, 1991). In addition, the fact that newborns usually have an increased haematocrit and that foetal erythrocytes contain more Hb per cell than those from adults (Delivoria-Papadopoulos and DiGiacomo, 1992) will further magnify this augmented ability of the blood in newborns to scavenge NO. It is thus implicit in the present study that, when compared with that of adults, blood in newborns would remove greater amounts of vasodilatory NO and consequently could facilitate the development of vasoconstrictory responses.

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