DILATOR ACTION OF Fe^{2+}-CITRATE COMPLEX ON THE RAT CAUDAL ARTERY PERFUSED IN VITRO

I. S. Kurbanov, N. A. Medvedeva, P. I. Mordvintsev, and A. F. Vanin

UDC 615.31:546.72].015.4:612.13].076.7

Key words: endogenous factors; iron; caudal artery.

In the modern view the dilator effect in blood vessels under the influence of certain endogenous and exogenous factors (acetylcholine, bradykinin, atrial peptide, nitroprusside, etc.) is initiated by activation of guanylate cyclase (GC), an enzyme responsible for the accumulation of cyclic guanosine monophosphate in cells [11, 14]. Free radicals (FR) of unsaturated fatty acids (UFA) or their peroxides can act as endogenous activators of GC [5, 10]. The so-called endothelium-dependent dilating factor (EDDF), isolated from endothelial cells of vessels under the influence of cholinergic and other agents [7, 12], also is similar in nature, according to most investigators [7, 12]. It can accordingly be postulated that dilatation of vascular smooth muscles can be induced by stimulating the accumulation of FR and peroxides of UFA in them. In the investigation described below, bivalent iron in a citrate complex [15], known to possess pro-oxidative properties, was used as the agent inducing intravascular peroxidation of UFA.

EXPERIMENTAL METHOD

An isolated preparation of the rat caudal artery, perfused at constant flow rate, was used as the test object [2]. Rats (200-250 g) were anesthetized with urethane (1 g/kg intraperitoneally) and a segment 8-10 mm long was excised from the proximal part of the caudal artery and incubated for 40 min at 8-10°C in modified Krebs–Henseleit solution (in mM): NaCl, 118.0; KCl, 4.7; $CaCl_2$, 2.52; $MgSO_4$, 1.64; $NaHCO_3$, 24.88; KH_2PO_4, 1.18; glucose, 5.55; pH 7.4 [4]. The vessel was transferred into a constant-temperature (37°C) aerated (95% O_2 + 5% CO_2) chamber and left there for 40 min until the preparation stabilized. Continuous perfusion of the vessel was used, for which purpose its two ends were fitted on to cannulas (d_{ext} = 0.6-0.8 mm). In the course of the experiment the inner surface of the vessel (at a constant flow rate of 2.0-2.5 ml/min) and the incubation chamber (5 ml/min) were perfused independently by means of an LKB (Sweden) roller pump. The perfusion pressure developing in the vessel under these circumstances did not exceed 30 ± 2 mm Hg. Besides the pressure created by the perfusion system itself (10 mm Hg) the initial perfusion pressure averaged 40 ± 2 mm Hg. This pressure was not taken into account in subsequent calculations, for the vessel was in the maximally relaxed state, as shown by absence of a response to papaverin ($5 \cdot 10^{-6}$ g/ml). Pressure was measured by an electromanometer ("Statham," USA). The endothelium in the vessel was removed by blowing a jet of air or distilled water through it for 30 sec under a pressure of 40 mm Hg, after which its removal was verified physiologically and histologically. Electronparamagnetic resonance (EPR) signals from the frozen perfusion solution were recorded on "Brucker ER200" and "Varian E9" radiospectrometers. The following reagents were used: noradrenalin (NA) bitartrate, from "Sigma," USA; sodium nitroprusside, from "Reanal," Hungary; $FeSO_4 \cdot 7H_2O$ and sodium citrate were of the chemically pure grade. The Fe^{2+}-citrate ratio in the solution was 1/5 (pH 7.2). As water-soluble antioxidants we used phenosan (potassium salt of 3,5-di-*tert*-butyl-4-hydroxyphenylpropionic) and Ikhfan (acid salt of d,l-tartaric acid and N,N-dimethyl-4-hydroxy-3,5-di-*tert*-butylbenzylamine), synthesized at the Institute of Chemical Physics, Academy of Sciences of the USSR. Hemoglobin (Hb) was obtained from mouse red blood cells by the usual method, and its concentration in the solution was determined spectrophotometrically. A dinitrosyl complex of iron (DNCI) with thiosulfate was obtained by the method described

Institute of Chemical Physics, Academy of Sciences of the USSR, Moscow. Faculty of Biology, M. V. Lomonosov Moscow University. (Presented by Academician of the Academy of Medical Sciences of the USSR I. P. Ashmarin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 4, pp. 366-369, April, 1990. Original article submitted February 24, 1988.

 0007-4888/90/0004-0478$12.50

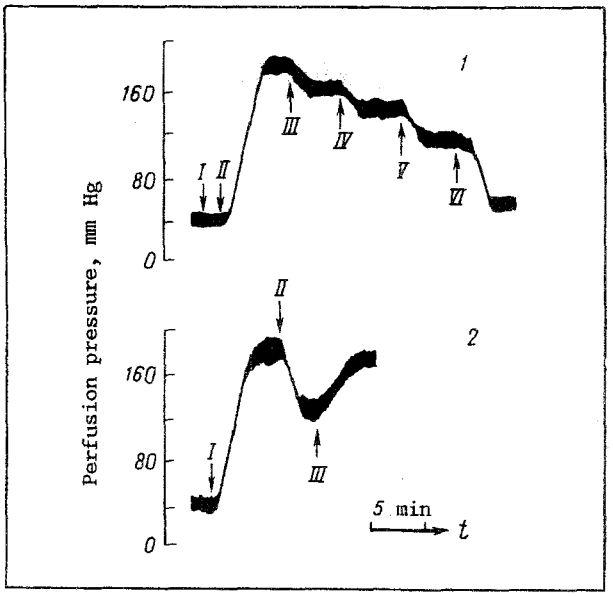

Fig. 1. Dilator effect of Fe^{2+}-citrate ($3 \cdot 10^{-7}$-$3 \cdot 10^{-4}$ M) on vessel (curve 1), action of hemoglobin ($4 \cdot 10^{-5}$ M) on this effect (curve 2).

in [1]. In some experiments bovine serum, and also mouse and human blood plasma, were used as the perfusion fluid. The experimental results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

To create the initial tone, the caudal artery preparation was perfused with NA solution in a concentration of 10^{-5}-10^{-6} g/ml, which caused the perfusion pressure to rise up to 180 ± 60 mm Hg (Fig. 1; Table 1). Additional Fe^{2+}-citrate ($3 \cdot 10^{-7}$-$3 \cdot 10^{-4}$ M) caused a dose-dependent decrease in the vascular tone induced by NA (Fig. 1). The maximal dilator effect was observed with Fe^{2+}-citrate in a concentration of $3 \cdot 10^{-4}$ M, when it averaged 84 ± 10% (Table 1). The dilator action of Fe^{2+}-citrate on the vessel was reversible. Citrate in the same concentration, without iron, had no appreciable dilator effect. Water-soluble antioxidants, namely phenosan and Ikhfan, in a concentration of 10^{-8}-10^{-9} M, themselves did not affect the degree of vascular tone, but abolished the dilator effect of Fe^{2+}-citrate virtually completely (Fig. 2). On average for 5 experiments the reduction of vascular tone under the influence of $3 \cdot 10^{-5}$ M Fe^{2+}-citrate was 80 ± 30 mm Hg ($p < 0.05$), whereas after the addition of phenosan it was 10 ± 10 mm Hg ($p < 0.05$). As another agent inhibiting peroxidation of UFA, Hb was used [15]. With Fe^{2+}-citrate in a concentration of $3 \cdot 10^{-7}$-$3 \cdot 10^{-6}$ M, Hb ($4 \cdot 10^{-5}$ M or above) abolished the dilator action of Fe^{2+}-citrate by 100% in all experiments ($n = 6$) (Fig. 1). The inhibitory action of Hb was not exhibited in the presence of Fe^{2+}-citrate in a concentration of 10^{-5} M (Fig. 1), despite the rise of the Hb concentration to $4 \cdot 10^{-4}$ M.

When physiological saline was replaced by serum or plasma the dilator action of Fe^{2+}-citrate was more marked. Incidentally, the dilator effect of this complex was independent of the presence of endothelium.

Thus the results of the experiments using synthetic antioxidants and Hb agree with views on the role of FR or peroxides of UFA in the dilator effect on vascular smooth muscles [5, 10]. Meanwhile it was shown by the EPR method that the blocking effect of Hb on vasodilatation was accompanied by the formation of paramagnetic nitrosyl complexes of this protein: in perfusion fluid containing $4 \cdot 10^{-4}$ M Hb an EPR signal was recorded which coincided in its parameters with EPR signal of the nitrosyl complex of Hb (Fig. 3). This result shows that vasodilatation induced by Fe^{2+}-citrate is accompanied by the production of nitric oxide in it. If it is recalled that this agent, as well as oxidation products of UFA, is a GC activator, and about ten times more effective [3, 9], it can be postulated that it is not FR or peroxides of UFA, but nitric oxide which activated GC in the vessel, thereby initiating its dilatation. Evidence that nitric oxide could in fact induce the dilator effect of this preparation is given by the results of experiments in which sodium nitroprusside or UFA with thiosulfate was added to the perfusion fluid as the source

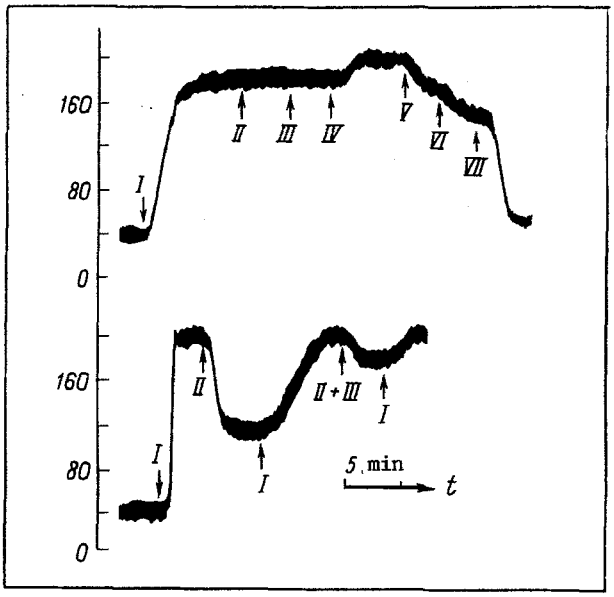

Fig. 2. Effect of phenosan ($5 \cdot 10^{-9}$-$5 \cdot 10^{-4}$ M) on vascular tone (curve 1) and its action on dilator effects of Fe^{2+}-citrate ($3 \cdot 10^{-5}$ M) (curve 2).

TABLE 1. Dilator Action of Fe^{2+}-Citrate during Perfusion of Caudal Artery with NA (10^{-5}-10^{-6} M) and Fe^{2+}-Citrate ($3 \cdot 10^{-4}$ M)

Expt. No.	Perfusion pressure, mm Hg		Reduction of perfusion pressure by action of Fe^{2+}-citrate	Abolition of vascular tone, %
	NA	NA + Fe^{2+}-citrate		
1	240	40	200	84
2	220	60	160	72
3	140	20	120	86
4	180	20	160	89
5	130	10	120	93
$M \pm m$	180±60	30±25	150±40	84±10

Legend. M) root mean square value, m) confidence interval at $p < 0.05$.

of nitric oxide [1, 9]. Both in a concentration of $5 \cdot 10^{-6}$ M reduced vasoconstriction induced by NA ($5 \cdot 10^{-6}$ M) by 100 and 70%, respectively.

We suggest the following mechanism of the dilator effect induced by Fe^{2+}-citrate in the vessel: this complex initiates the formation of FR or peroxides of UFA, and the latter oxidize endogenous amines of hydroxylamines with the formation of nitroso compounds, releasing nitric oxides, which activates GC and thereby initiates vasodilatation. This hypothesis is based both on the results described above and on those of an investigation in which marked activation of isolated soluble GC was recorded after its contact with a mixture of peroxide of linoleic acid and several organic amines and hydroxylamines [6]. It was shown that the corresponding organic nitroso compounds, which could produce nitric oxide, activating GC, were in fact produced in this mixture.

The formation of oxidation products of UFA under the influence of Fe^{2+}-citrate could take place both in the endothelium and in the smooth muscle cells themselves. In the present experiments the dilator action of this complex was found both in vessels containing endothelium and in those without it. A different situation can be realized under these circumstances for a dilator effect of the vessels that is dependent on endothelium and is induced, not by Fe^{2+}, but by cholinergic and other biogenic factors. In this case FR or peroxides of UFA, we consider, are formed only in the endothelium, and it is evidently they which are EDDF [7, 12]. This factor, on coming into contact with vascular smooth muscles, can oxidize endogenous amines and hydroxyl-

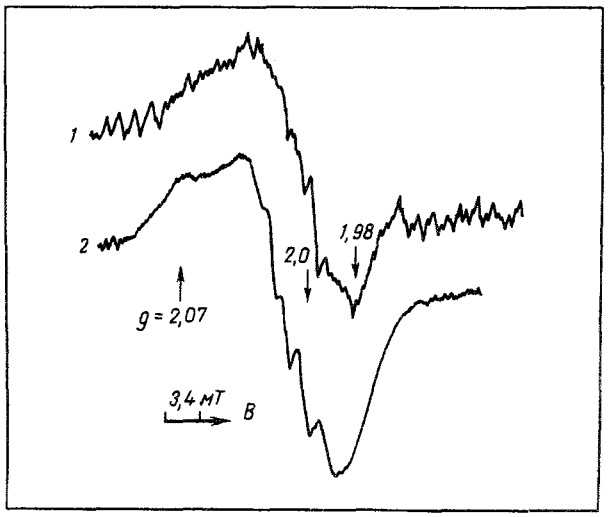

Fig. 3. EPR signals: 1) of perfusion fluid containing NA (10^{-5} M) + Fe^{2+}-citrate ($3 \cdot 10^{-6}$ M) + hemoglobin ($4 \cdot 10^{-4}$ M), after passage through vessel; 2) of Hb complex with NO. Recorded at 77 K.

amines in them, with the formation ultimately of nitric oxide, which induces the dilator effect. Preliminary experiments carried out in our group by L. V. Bezhikina, to study endothelium-dependent dilatation of a ring of rat aorta, initiated by acetylcholine, confirm this hypothesis, for the formation of nitrosyl complexes of heme and nonheme iron was recorded in the incubation medium by the EPR method.

It has been suggested that EDDF is nitric oxide [8], but this contradicts data obtained by other workers who demonstrated the selectivity of action of EDDF: it caused dilatation only of vascular smooth muscles, whereas nitric oxide had a dilator action on smooth muscles not only of blood vessels, but also of other organs [13].

LITERATURE CITED

1. A. L. Kleshchev, P. I. Mordvintsev, and A. F. Vanin, Stud. Biophys., **105**, 93 (1985).
2. N. A. Medvedeva, Wo T'ueh-chin, I. Yu. Sergeev, and I. M. Radionov, Byull. Éksp. Biol. Med., No. 5, 517 (1987).
3. W. P. Arnold, C. K. Mittal, C. Katsuki, and F. Murad, Proc. Nat. Acad. Sci. USA, **74**, 3203 (1977).
4. R. E. Blattner, H. C. Classon, H. Dehnert, and H. J. Döring, Hugo Sachs Elektronik (1978).
5. E. Böhme, R. Gerzer, G. Crossman, et al., Hormones and Cell Regulation, Amsterdam (1983), pp. 147-161.
6. F. R. De Rubertis and P. A. Graven, Biochem. Biophys. Res. Commun., **88**, 469 (1979).
7. R. F. Furchgott and J. V. Zawadsky, Nature, **288**, 373 (1980).
8. R. F. Furchgott, M. T. Khan, and D. Jothianandan, Fed. Proc., **46**, 385 (1987).
9. R. Gerzer, F. Hoffman, and G. Schultz, Eur. J. Biochem., **116**, 479 (1982).
10. H. Hidaka and T. Asano, Proc. Nat. Acad. Sci. USA, **74**, 3657 (1977).
11. F. Murad, J. Clin. Invest., **78**, 1 (1986).
12. R. M. Rapoport and F. Murad, Circulat. Res., **52**, 352 (1983).
13. K. Shikano and B. A. Berkowitz, Fed. Proc., **46**, 386 (1987).
14. C. Walter, "Protein phosphorylation," Adv. Cyclic. Nucl. Res., **17**, 349 (1984).
15. E. D. Wills, Biochim. Biophys. Acta, **98**, 352 (1965).