MEASUREMENT OF THE CONTENT OF Fe(III)—DESFERAL COMPLEXES IN THE PERFUSED RAT LIVER BY AN EPR METHOD

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An important role in the pathogenesis of several diseases is played by disturbances of intracellular iron metabolism [1, 2, 5-9]. A number of medicinal preparations are available now which have the ability to chelate iron ions. The best known of them is desferrioxamine (Desferal) [8, 10, 12]. To estimate the efficacy of binding of intracellular iron by this preparation, a method of determining the quantity of iron chelated within cells must be used.

Many complexes including Fe(III), for example, transferrin [4] and EDTA-iron [11], are known to be paramagnetic and to have an EPR spectrum in the g = 4.3 region. An important advantage of the EPR method over optical methods is that measurement can be made in tissues without their preliminary homogenization.

The aim of this investigation was accordingly to study the possibility of using the EPR method to assess the quantity of complexes of intracellular iron with desferal in the tissues.

EXPERIMENTAL METHOD

Male Wistar rats weighing 200-250 g were used. The liver was perfused with Hank's solution, with a closed circuit, under ether anesthesia. The perfusion fluid passed from the circuit into the liver via the portal vein, and from the liver back to the circuit via the inferior vena cava. A clamp was applied to the superior vena cava. Fluid circulated in the circuit on account of a peristaltic pump and it was oxygenated with pure oxygen, with continuous monitoring of its concentration in the fluid flowing from the liver (pO_2 was 50-70 mm Hg). The rate of perfusion was 9 ml/min.

The commercial product desferal (Ciba) was used, and all reagents of Soviet origin were of the chemically pure grade.

Optical absorption spectra were recorded on a Beckman DU-7 spectrophotometer, within the 300-600 nm range. Specimens for EPR spectrometry were prepared as described previously [3]. EPR spectra in the g = 4.3 region were recorded at the temperature of liquid nitrogen on a Varian E-4 spectrometer, under the following conditions: amplification 2.5×10^3; amplitude of modulation 16 G; scanning speed of spectrum 250 G/min; power 10 mW. A base line was drawn on the EPR spectrum and the amplitude of the low-field component (A_L) and the amplitude of the high-field component (A_H, Fig. 1) were measured relative to it. The number of paramagnetic centers was judged by the amplitude of the signal $A = A_L + A_H$.

EXPERIMENTAL RESULTS

Desferal forms a colored complex with Fe(III) with absorption maximum at 430 nm. By determining the optical density (D_{430}) the concentration of desferal—iron complexes in a test solution can be judged, and later, after the addition of an excess of Fe(III) ions to the solution (in this case the molar ratio of $FeCl_3$:desferal = 20:1), the quantity of desferal not bound with iron can be judged by the increase in D_{430} relative to its initial level. By using this approach, the concentration of desferal—iron complexes and of free desferal in the perfusion fluid, obtained by perfusion of the liver with Hanks' solution containing 0.5 mM

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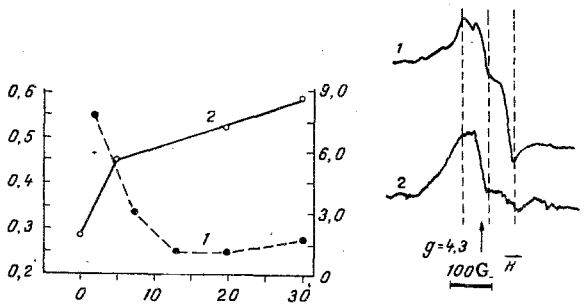

Fig. 1. Concentration and absorption spectrum of desferal–Fe(III) complexes. Concentration of desferal in perfusion medium estimated from D_{430} (1), and concentration of desferal–iron complexes in liver, estimated from amplitude of EPR signal (2) during perfusion of liver with Hanks' solution containing 0.5 mM desferal.

Fig. 2. EPR signal of perfused liver in g = 4.3 region (2) and EPR signal of blood serum transferrin (1).

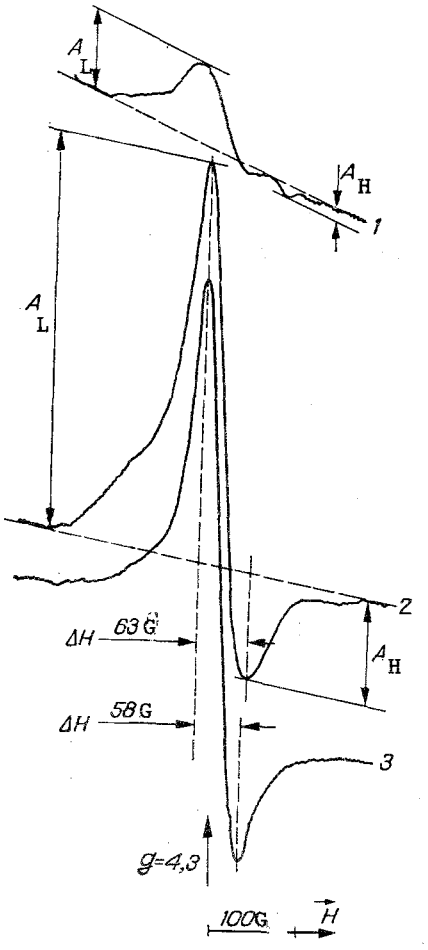

Fig. 3. EPR signal of liver perfused in presence (2) and absence (1) of desferal. EPR signal of desferal–Fe(III) complex (3).

TABLE 1. Amplitude of EPR Signal with g = 4.3 in Liver Perfused with and without Desferal, Relative Units (M ± m)

Conditions of perfusion	Before perfusion	After 30 min of perfusion
Without desferal	1,3±0,2	1,1±0,4
With desferal	1,3±0,2	8,7±1,4

desferal, was determined. In the course of 30 min no desferal–iron complexes were found in the perfusion fluid. The concentration of desferal not bound with iron in this case fell sharply during the first 10 min after the beginning of perfusion, and then remained virtually unchanged (Fig. 2).

It can be postulated that desferal passes from the perfusion medium into the liver cells, where it chelates Fe(III) ions. As was pointed out above, complexes of Fe(III) are characterized by an EPR signal in the g = 4.3 region. Accordingly, we studied the EPR spectrum of the liver, when perfused with desferal and without it.

Recording the EPR spectrum of the liver perfused without desferal showed the presence of a signal of low intensity in the g = 4.3 region, with a half-width (ΔH) of about 125 G. This signal was similar in shape to the EPR spectrum of serum transferrin (Fig. 3). It can be tentatively suggested that the signal observed either belongs to serum transferrin, not flushed out of the vascular bed during perfusion, or to transferrin taken up by hepatocytes through pinocytosis, and located inside them. Perfusion of the liver with Hanks' solution for 30 min did not lead to any significant change in amplitude of this signal (Table 1). No such signal could be found in the perfusion fluid.

After perfusion of the liver for 30 min with Hanks' solution containing 0.5 mM desferal, an EPR signal with g = 4.3 was recorded in it. The half-width of the signal was 63 G. The amplitude of the signal was more than 7 times greater than that of the signal from native liver (Table 1). A signal similar in its position in the spectrum and similar in shape (g = 4.3; ΔH = 58 G) was recorded in an aqueous solution containing Fe(III) ions and desferal (Fig. 1). Hence it can be concluded that in the case of the tissue we were dealing with the EPR signal of a complex formed by binding of intracellular free iron Fe(III) with desferal, entering the cell from the perfusion solution.

Perfusion of the liver with Hanks' solution containing desferal for 5, 20, and 30 min showed that the majority of desferal–iron complexes are formed during the first 5 min of perfusion (Fig. 2). During the next 25 min the number of complexes increases, but more slowly. This is in good agreement with the data in Fig. 2, from which it is clear that most of the desferal passes from the perfusion fluid into the liver during the first 10 min of perfusion.

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FAST CALCIUM ENTRY ACTIVATION IN DENERVATED SMOOTH MUSCLE OF THE CAT NICTITATING MEMBRANE

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Severing the connection between efferent neurons and the target cell leads to structural and functional changes and, in the case of smooth-muscle cells (SMC), to changes in the contractile system and excitable membrane. Similar changes are found in tonic fibers of striated muscle after denervation [5, 9]. In SMC, just as in many other objects (neurons, glands, striated muscle) denervation leads to increased sensitivity to neurotransmitters and hormones [8]. It was shown previously [1] that, besides causing a shift of the dose — effect curve and of expression of the contractile and membrane response, the denervated SMC of the cat nictitating membrane can generate action potentials (AP). Generation of agonist-mediated AP is not characteristic of innervated SMC of this particular object, and this makes them similar to SMC of large arteries, whose work is concerned with pharmacomechanical coupling between excitation and contraction (E–C). Meanwhile we know that a large group of SMC (in the intestine and ureter) generate AP in the innervated state and have an electromechanical type of E–C coupling [3, 6]. Denervation evidently leads to a change not only of sensitivity, but also of E–C coupling. This explains the importance of a study of the ionic mechanisms of AP generated in SMC with the pharmacomechanical type of E–C coupling after denervation.

EXPERIMENTAL METHOD

Experiments were carried out on the inferior smooth muscle of the cat nictitating membrane, isolated in noninbred animals weighing 2.5 kg. A surgical denervation model was used. After isolation of the nictitating membrane, the lower layer of the smooth muscle was carefully dissected free from the connective tissue and cut into strips 20 mm long and 0.5 mm wide. After isolation the strips were incubated in Krebs' solution (in mM): NaCl 120, KCl 5.9, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $NaHCO_3$ 15.5, glucose 11.5 (pH 7.4); 95% O_2 and 5% CO_2. Contraction was recorded by a mechanical to electrical transducer of original design [2]. An isotonic sucrose solution was prepared by dissolving chemically pure sucrose in bidistilled water, and hyperpotassium solutions were prepared by adding the dry salt to Krebs' solution. Calcium-free solutions contained 2 mM EGTA and 10 mM $MgCl_2$ to stabilize the cell membrane. Replacement of calcium by barium was carried out on an equimolar basis. Sodium was replaced by Tris-HCl. In experiments to study the effect of inorganic calcium blockers, the phosphate-bicarbonate buffer was replaced by HEPES (10 mM). Noradrenalin (NA) was obtained from Merck, West Germany, nifedipine from Arzneimittelwerke, East Germany, and Tris and HEPES from Sigma, USA.

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