EFFECT OF ADRENALIN ON THE FREE RADICAL LEVEL IN HUMAN PLASMA AND ERYTHROCYTES

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Data in the literature [8] are evidence that catecholamines and, in particular, adrenalin affect the development of attacks of angina in patients with ischemic heart disease (IHD). The writer found an increase in the free radical (FR) concentration in the erythrocytes and blood of patients with IHD whether attacks of pain in the region of the heart were present or not. During an attack the FR concentration in the plasma rises. The FR signal in erythrocytes with a g value of about 2.0030-2.0040 and with ΔH about 12-15 Oe belongs to the flavosemiquinone glutathione reductase, whereas the signal in the plasma with $g \sim 2.0024$-2.0029 and $\Delta H \sim$ 6-8 Oe belongs to cations of polycyclic hydrocarbons [6].

Ceruloplasmin (CP) is known to oxidize biological amines in vitro [1]. However, since the maximal reaction velocity of oxidation of these substrates by CP in vitro is observed at pH 5.5 and since it is reduced at pH values between 7.0 and 7.5, the biological importance of oxidation of these substances by CP is disputed by some workers [10].

There are no data in the literature on the effect of adrenalin on the FR and CP level in human erythrocytes and plasma, and the investigation described below was accordingly carried out to remedy this omission.

EXPERIMENTAL METHOD

Human blood with heparin was taken from the cubital vein and centrifuged at 1500 rpm for 10 min. The plasma was separated from the erythrocytes and centrifuged at 3000 rpm for 5 min (to sediment leukocytes). It was then frozen in liquid nitrogen in Teflon molds. The erythrocytes were washed 3 times with cold physiological saline (1:10) and also frozen in liquid nitrogen after preparation [6]. Erythrocytes and plasma which remained unfrozen (pH 7.36) were treated with 0.01 ml of adrenalin hydrochloride solution and incubated at 25°C for 30 min. After incubation they also were frozen in liquid nitrogen. The erythrocytes and plasma were expressed from the molds for examination, placed in a quartz Dewar flask, and incubated. Recordings were made with a "Rubin" EPR spectrometer. Mn^{++} in an MgO lattice was used as the side standard. The number of paramagnetic centers (PC) was determined in relative units from the ratio of the amplitude of the signal from the recorded specimen and the amplitude of the signal from the side standard.

EXPERIMENTAL RESULTS

The following PC were determined by analysis of human plasma and erythrocytes by the EPR method. A signal was found in plasma with a g factor of ~ 4.26 (Fig. 1), due [9] to the high-spin form of Fe^{+++}, with nonhemin iron complex, belonging to the transferrin Fe^{+++} signal [12]. EPR spectroscopy of the plasma also revealed a signal with g factor of ~ 2.05. This signal was due to Cu^{++} atoms present in the protein composition of CP [12]. The signal of FR with $g \sim 2.0024$-2.0029 and ΔH 6-8 Oe also was recorded in plasma. It is suggested that this signal was due to unpaired electrons delocalized as regards protein structure, or to ions of polycyclic hydrocarbons [2].

A signal with $g \sim 6.00$ (Fig. 2), due to the presence of a high-spin form of hemoproteins and belonging to hemoglobin [11], was found in the erythrocytes. A signal with $g \sim 2.063$ belonging to superoxide dismutase was present [13]. A signal of FR with $g \sim 2.0030$-2.0040 and ΔH 12-15 Oe also was recorded in the erythrocytes.

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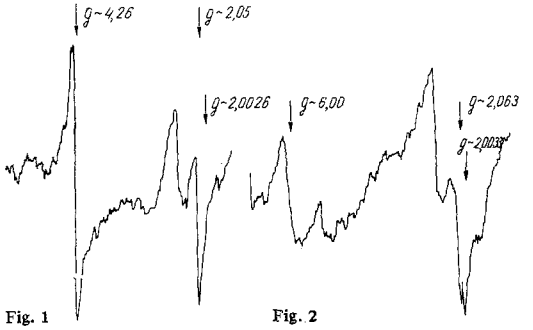

Fig. 1 Fig. 2

Fig. 1. EPR signals of plasma at 77°K. Conditions of recording, here and in Fig. 2: H_0 = 2300 Oe, ΔH = 3200 × 1.4, scanning speed 500 Oe/min, sensitivity 100, modulation 10 Oe, time constant 0.3, power of SHF radiation 10 dB.

Fig. 2. EPR signal of erythrocytes at 77°K.

TABLE 1. Effect of Adrenalin on PC Level in Human Plasma and Erythrocytes (M ± m)

PC	Experimental conditions	Number of PC, relative units	
		plasma	erythrocytes
Cu^{2+}	Control	151,64±10,0	70,79±11,5
	Adrenalin	101,52±8,24	64,52±6,57
		$P<0,01$	$P>0,05$
FR	Control	22,0±2,30	49,0±5,50
	Adrenalin	32,0±3,40	122,15±13,40
		$P<0,05$	$P<0,001$

Addition of 0.01 ml of a 0.1% solution of adrenalin to the plasma and erythrocytes, followed by incubation for 30 min at 25°C, caused changes in the PC concentration (Table 1).

A fall in the Cu^{++} level and a rise in the FR concentration were observed in plasma. In erythrocytes the Cu^{++} concentration was virtually unchanged but the FR level rose.

The results show that the CP signal in plasma was reduced by 49.4 whereas at the same time the FR level rose by 41.4. This was evidently due to reduction of Cu^{++} into Cu^{+} and simultaneous oxidation of adrenalin and the formation of an adrenalin FR. The adrenalin FR may perhaps form an enzyme–substrate complex with CP, as some workers have suggested [14], or the adrenalin FR hands over its unpaired electron to a protein or to the ion of a polycyclic hydrocarbon.

The possibility cannot be ruled out that this mechanism also operates in vivo in response to the release of large quantities of catecholamines into the blood stream. This evidently also explains the rise in the FR level found in plasma of patients with IHD during an attack of angina.

A considerable rise (by 149.2%) in the concentration of FR with g ~ 2.0030-2.0040 and ΔH ~ 12-15 Oe was found in the erythrocytes. Adrenalin is known to affect certain biochemical processes in tissues and organs [4]. The writer showed previously that FR in erythrocytes are due to the semiquinone FAD, a coenzyme of glutathione reductase [6]. An increase in the quantity of reduced forms of NADP and NAD was demonstrated in [3] under the influence of adrenalin. NADPH is the substrate for glutathione reductase during reduction of glutathione. Under these circumstances glutathione reductase is activated, the content of FAD semiquinone increases, and the FR concentration rises. The possibility cannot be rule out that adrenalin is catabolized along the quinone path with the formation of oxo-derivatives, which inhibit SH groups [7]. The glutathione reductase system is known [5] to participate in the reduction of sulfhydryl groups, and for that reason when these groups are blocked glutathione reductase is activated, and oxidized glutathione is converted into reduced glutathione. The latter promotes reduction of sulfhydryl groups.

It can thus be concluded that adrenalin is one of the substances which can raise the FR level in the plasma and that it can activate the glutathione reductase system in human erythrocytes.

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IMMUNOCHEMICAL IDENTIFICATION AND PHYSICOCHEMICAL CHARACTERISTICS OF SPECIFIC PROTEINS OF SEMINAL PLASMA

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Investigation of the antigens of seminal plasma is particularly important in order to characterize the functional state of the reproductive system and of the blood–testis barrier and also to evaluate any possible immunological causes of sterility. Seminal plasma contains normal blood serum proteins (albumin, immunoglobulins) [11, 12] and also certain unidentified antigens [7, 8]. The present writers found placental α_2-microglobulin in sperm [3]. It is suggested [9, 10] that the study of sperm proteins and their exposure to various factors is the most specific method of verification of male fertility.

The aim of the present investigation was to seek specific proteins in human seminal plasma and to study them.

EXPERIMENTAL METHOD

To obtain antisera rabbits were immunized with pooled healthy human seminal plasma. The antisera were exhausted with dry blood plasma obtained from donors. The schemes of immunization, and methods of preparation of the tissue extracts and of determination of the physicochemical parameters of the proteins were described by the writers previously [2, 5]. Antigens were determined semiquantitatively by the double immunodiffusion method with a standard test system [6]. Immunoelectrophoresis was carried out in 1% agar gel, from Serva (West Germany).

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