EFFECT OF DIAZEPAM ON PROPERTIES OF THE ERYTHROCYTE–PLASMA PROTEINS COMPLEX AND THE DEVELOPMENT OF SHOCK AFTER HETEROLOGOUS BLOOD TRANSFUSION

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Preliminary injection of 2 mg/kg diazepam abolishes the pyrogenic reaction and increases the survival rate of rabbits after intravenous injection of cat blood. A study of the physicochemical properties of the blood by measurement of the ionization equilibrium during thermal denaturation showed that injection of heterologous blood into rabbits and suspending rabbit's erythrocytes in cat's plasma in vitro lead to a sharp decrease in the rate of proton binding by the plasma protein in the presence of the foreign erythrocytes. Preliminary injection of diazepam to the rabbits and addition of diazepam to the cat's plasma in vitro prevent these changes and enable the blood to retain its native properties. Addition of diazepam in a concentration of about 10^{-4} M to heterologous plasma considerably reduces erythrocyte agglutination.

KEY WORDS: diazepam; transfusion shock; erythrocytes; plasma.

Benzodiazepine derivatives are used in clinical practice mainly as tranquilizers and anticonvulsants [2]. However, recently they have been shown to have a marked antihistamine, antiserotonin, and antibradykinin action [7, 8]. Preparations of this class have been found to have antiedematous and antiinflammatory properties [4]. These compounds have been shown to be effective in anaphylactic shock, bronchial asthma, urticaria, and rhinitis [5, 6, 8]. These facts are evidence that benzodiazepine derivatives inhibit reactions of increased sensitivity of immediate and delayed types and have a marked effect on the immune system of the body.

Considering that the search for substances effective against incompatibility reactions is a very urgent problem, in this investigation the effect of diazepam on the development of shock and on some physicochemical properties of the erythrocytes and plasma was studied after intravenous injection of heterologous blood into rabbits.

EXPERIMENTAL METHOD

Experiments were carried out on 30 rabbits. All the animals received an intravenous injection of fresh heparinized cat's blood in a dose of 2-3 or 6-7 ml/kg. One group of animals was given diazepam (Seduxen, in ampuls, from Richter, Hungary) in a dose of 2 mg/kg intramuscularly 30 min before the transfusion of heterologous blood. The O_2 uptake and CO_2 excretion (by the Spirolit apparatus), the rectal temperature, and the blood pressure (by a direct method) were measured in the rabbits. The physicochemical properties of the blood were assessed from its ionization equilibrium during thermal denaturation [3]. The same method was used to study the properties of a mixture of erythrocytes and heterologous plasma in vitro. Changes in the pH of the plasma, erythrocytes, and whole blood during elevation of the temperature (58°C) were recorded continuously by the 262 pH-meter with the aid of an ÉSL-63-07 electrode and KSP-4 automatic writer.

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TABLE 1. Changes in Rectal Temperature, Basal Metabolism, and Value of tan α for Rabbits after Intravenous Injection of 2-3 ml/kg Cat's Blood (M ± m)

time, min	Without diazepam				Diazepam, 2 mg/kg			
	T,°C	CO_2, %	O_2, %	tan α	T, °C	CO_2, %	O_2, %	tan α
0	38,0±0,1	100%	100%	0,84±0,12	38,1±0,1	100%	100%	0,98±0,14
15	39,2±0,3*	172±8*	148±7*	0,19±0,08*	37,9±0,1	98±2	100±5	0,91±0,15
30	39,7±0,1*	198±5*	170±8*	0,16±0,08*	37,7±0,2	105±4	102±2	0,84±0,10
45	40,3±0,1*	162±5*	151±10*	0,21±0,07*	37,9±0,2	106±7	103±5	0,98±0,13
60	40,6±0,2*	137±11	134±14*	0,31±0,10*	38,3±0,2	104±2	102±4	0,92±0,07

Note. Values differing statistically significantly from initial values ($P < 0.05$) indicated by asterisk.

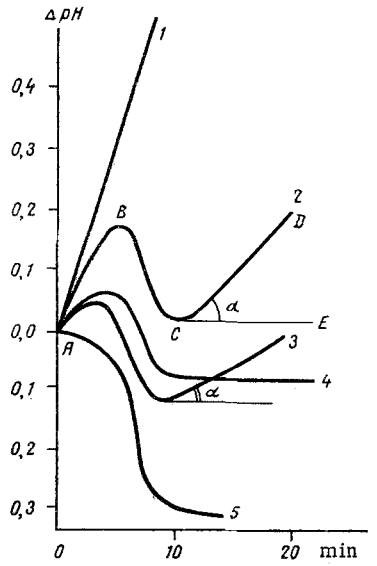

Fig. 1

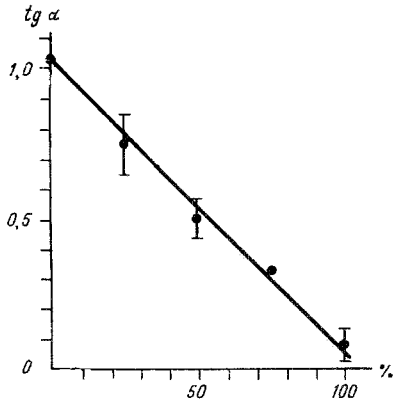

Fig. 2

Fig. 1. Changes in pH of rabbit's plasma (1), rabbit's whole blood (2), washed rabbit's erythrocytes (5), and rabbit's erythrocytes in cat's plasma in the presence of diazepam (3) and in its absence (4) at 58°C.

Fig. 2. Dependence of tan α on quantity of cat's plasma in rabbit's blood. Abscissa, quantity of cat's plasma (in % of rabbit's plasma).

EXPERIMENTAL RESULTS

The results of the in vivo experiments are given in Table 1. In all the rabbits, after intravenous injection of 2-3 ml/kg of cat's blood the rectal temperature rose by 2-3°C and the O_2 uptake and CO_2 excretion were increased. In the animals receiving a preliminary injection of diazepam no such changes developed. In most rabbits which were given an injection of 6-7 ml/kg of cat's blood after diazepam the blood pressure was unchanged, whereas in the animals not receiving diazepam it fell sharply. All rabbits not receiving diazepam died in the course of 2 weeks, whereas all the animals receiving diazepam survived after the smaller dose of heterologous blood and 40% survived after receiving a larger dose of blood.

Denaturation of the blood plasma at 58°C in vitro was accompanied by proton binding which obeyed a linear relationship (Fig. 1), whereas the erythrocytes gave up protons in accordance with a S-shaped curve. In other words, erythrocytes are proton donors and plasma a proton acceptor. For whole blood of intact animals the curve of the change in affinity of the protons during exposure to heat is the geometric sum of curves 1 and 5; proton binding in regions AB and CD, moreover, is connected with the ability of plasma to bind protons, whereas the decrease in pH in the region BC is connected with the giving up of protons by the erythrocytes. However, this geometric summation is characteristic only for plasma and erythrocytes of animals belonging

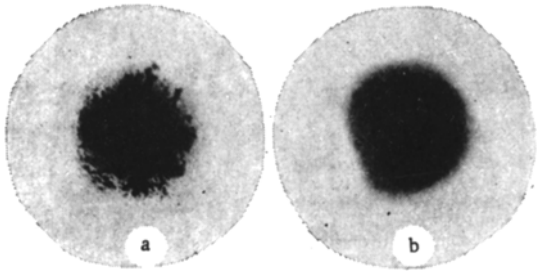

Fig. 3. Rabbit's erythrocytes in cat's plasma without (a) and with (b) diazepam in a concentration of 10^{-4} M.

to the same species. On mixing plasma and erythrocytes of animals of different species the shape of the curve may differ considerably. For instance, when rabbits' erythrocytes were suspended in cat's plasma curve 4 was recorded without a rise in the region CD, although cat's plasma is just as good a proton acceptor as is rabbit's plasma. The presence of heterologous erythrocytes thus led to loss of the acceptor properties by the plasma. To assess the changes in the physicochemical properties of the blood quantitatively on mixing heterologous erythrocytes and plasma, the value of tan α was used. Gradually increasing the proportion of cat's plasma in rabbit's blood in experiments in vitro (washed rabbit's erythrocytes were suspended in a mixture of rabbit's plasma and cat's plasma) led to a virtually linear decrease in the value of this index (Fig. 2).

Injection of heterologous blood into rabbits was accompanied by a considerable decrease in the value of tan α, whereas in animals receiving diazepam tan α was unchanged (Table 1). Preliminary addition of diazepam in a concentration of 10^{-4} M to cat's plasma in vitro also led to restoration of the acceptor properties of the plasma in the presence of rabbit's erythrocytes (Fig. 1).

Special experiments showed that diazepam, in a concentration of $5 \cdot 10^{-5}$–$1 \cdot 10^{-4}$ M prevents agglutination of rabbit's erythrocytes in cat's plasma (Fig. 3). Similar results also were obtained with human blood of different groups: Preliminary addition of diazepam to serum in a concentration of about 10^{-4} M considerably prevented agglutination of erythrocytes of groups A and B suspended in the corresponding hemagglutinating serum.

The results of the present investigation indicate that diazepam has a substantial effect on the immune reactivity of the animal following injection of foreign blood, by intervening directly in the interaction between erythrocytes and plasma protein. The writers previously [3] postulated the structural differentiation of native blood. Mixing incompatible erythrocytes and plasma evidently leads to disturbance of the supramolecular structures of the liquid part of the blood, ruptures the bonds stabilizing the erythrocyte–plasma proteins complex and, ultimately, leads to agglutination of the erythrocytes. Considering that the pathogenesis of shock following transfusion of heterologous blood is based on a disorder of the blood flow caused by agglutination [1], the protective action of diazepam in the present experiments can be attributed to its ability to prevent this disturbance of the native properties of blood.

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