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THE EFFECTS OF β-DIETHYLAMINOETHYL-DIPHENYLPROPYLACETATE (SKF 525-A) ON BIOLOGICAL MEMBRANES—I.

SKF 525-A-INDUCED STABILIZATION OF HUMAN ERYTHROCYTES*

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Abstract—β-Diethylaminoethyl-diphenylpropylacetate (SKF 525-A) results in either hemolysis or stabilization of the red cell membrane. These effects are concentration dependent. At higher concentrations of SKF 525-A (10^{-3}M), an increase in hemolysis and a decrease in osmotic resistance is noted, whereas a stabilization of the red cell membranes occurs at lower concentrations of 10^{-4}–10^{-9}M. The hemolysis of the red cell is temperature dependent and hemolysis increases as temperature decreases. The SKF 525-A action appears to be immediate and the effects prolonged. The concentration at which SKF 525-A demonstrates its greatest membrane stabilization corresponded to the first breaking point observed during measurements of surface tension, and thus perhaps indicates the formation of micelles. It can be calculated that one molecule of SKF 525-A occupied 51 Å^2 of red cell area. SKF 525-A (10^{-4} M) causes a change in the mean cellular volume of red cells over all osmolar concentrations studied. One possible mechanism of action that would account for these findings is that the erythrocyte membrane expands in combination with SKF 525-A in the same way that lipid monolayers interact with various surface-active drugs such as the phenothiazine derivatives. The resulting expansion of the erythrocyte membrane would lead to an increase in the surface area and volume of the red cell and thus decrease osmotic fragility.

β-DIETHYLAMINOETHYL-diphenylpropylacetate HCl (SKF 525-A) is an inhibitor of a wide variety of hepatic microsomal drug biotransformations.[1] SKF 525-A also inhibits the biosynthesis of proteins[2] and cholesterol.[3] The mechanism of enzyme inhibition is for the most part noncompetitive[4] and reported effects are immediate and prolonged.[5]

Several mechanisms of action have been theorized to explain the inhibition of such diverse enzymatic reactions. Netter[4] proposed that because the NADPH-dependent oxidase system is common to all oxidative reactions, SKF 525-A may act as an uncoupling agent. However, SKF 525-A also inhibits non-NADPH metabolic reactions, for example nitro reductase[6] and glucuronyltransferase[7] reactions. Fouts[8] has suggested that there is a correlation between endoplasmic reticulum structure and the levels of activity of certain enzymes in the microsomes. Brodie[9] has suggested that SKF 525-A may have a physicochemical effect on the microsomal membrane to alter

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its permeability to drugs. Hollunger,[10] based on his microsomal amidase studies, reported that SKF 525-A acts on the enzymes *per se* rather than on the microsomal membrane. Anders and Mannering[11] have suggested that microsomal demethylase is competitively inhibited by SKF 525-A. It is therefore obvious that the mechanism of action of SKF 525-A has yet to be totally elucidated.

This paper presents data obtained during an investigation of the relationship between the physicochemical properties of SKF 525-A and its effect on the osmotic resistance of human red cells. It was felt that the red blood cell membrane perhaps offered a tool by which the action of SKF 525-A on biological membranes could be further elucidated.

METHODS

Preparation of human red blood cell suspension. Human blood from healthy donors was collected into screw-cap vials containing 100 units of sodium heparin per 10 ml whole blood. Shortly after the collection, the heparinized blood was transferred to 40-ml graduated conical centrifuge tubes and centrifuged at 200 *g* at room temperature in an International centrifuge with horizontal head. The plasma and buffy coat of white blood cells were carefully removed by gentle aspiration. The red cells were resuspended in 150 mM NaCl by gentle inversion of the centrifuge tube. The platelets were removed by centrifuging at 100 *g*, and the red cells were washed three times with 150 mM NaCl. The microhematocrit of the final suspension was determined with an Adams Autocrit centrifuge. The final suspension was adjusted to provide a hematocrit of about 50 per cent. The number of red cells in the stock suspension, determined by a Coulter electronic cell counter, ranged from 4·5 to 4·9 $\times$ 10^6 cells/mm^3. Since the normal range of 50 per cent hemolysis in normal adults is between 136 and 150 milliosmols, it was necessary to determine the per cent hemolysis by using the same cell population throughout each individual experiment. Exceptions to this procedure are noted in the text.

Preparation of varying osmolarity of NaCl solution. Baker's analyzed reagent grade NaCl, KCl, NaH_2PO_4–H_2O and Na_2HPO_4 were placed in a mechanical convection oven overnight at 50°; they were then removed from the oven and immediately placed in a desiccator over Silica gel for 1 hr before the chemicals were weighed. In order to compare the pH effect of SKF 525-A HCl-altered osmotic resistance of the red cells, two different test solutions were prepared according to Mortensen[12] and Emerson *et al.*[13] The prepared test solutions ranged from 300 to 68 mOsmole. The pH of the buffered and unbuffered test solutions were 7·36 to 7·45 and 6·0 to 6·5 respectively. The final pH of the test solutions after adding either SKF 525-A HCl or chlorpromazine HCl is indicated in each of the experimental results. Hemolysis and osmotic resistance of red cells were determined by the quantity of hemoglobin released.

The amount of hemoglobin released from the red cells was determined colorimetrically. The experiments were carried out as follows: 0·1 ml of stock red cell suspension was added to 1·0 ml of test solution containing either 0·1 ml of test compounds or 0·1 ml of distilled water in a 10 $\times$ 75 mm Kimble disposable culture tube and immediately mixed by gentle inversion of the tube stoppered with parafilm. Most of the tests were performed with 150 mOsmole NaCl except when a total range of varying osmolarity was used to check whether red cell samples fell within the normal range of the osmotic fragility curve. The mixture of the test sample was

incubated at varying temperatures and also for varying times. At the end of the incubation time, tubes were centrifuged at 500 *g* for 15 min with an International centrifuge model K with horizontal head. The supernatant fraction was carefully transferred to another disposable culture tube by a disposable pipette (Clay-Adams Transpets, 9 in. long) so as not to disturb packed red cells. The supernatant (0·5 ml) was diluted with ammoniated distilled water (0·05 ml of 30% NH_4OH per 10 ml distilled water) in either 12 × 75 mm or 19 × 150 mm round Coleman cuvettes. Extinction of hemoglobin at 540 mμ was determined with a Coleman Jr. II spectrophotometer. All of the experimental samples were studied in either duplicate or quadruplicate.

Surface tension measurements of SKF 525-A HCl and chlorpromazine HCl. Surface activities of varying aqueous concentrations of SKF 525-A HCl and chlorpromazine HCl were determined at 23° by means of the Wilhelmy plate method.[14] The Wilhelmy plate apparatus, precision Rosano surface tensiometer (model ST 0500 MG), was used for all the surface tension measurements. Varying concentrations of both SKF 525-A HCl and chlorpromazine HCl, ranging from 10^{-2}–10^{-7} M were dissolved in the following solutions: 150 mOsmole NaCl (pH 5·3), 150 mOsmole NaCl-HCl (pH 2·00) and also 300 mOsmole NaCl (pH 5·70). The objective of dissolving these compounds in varying ionic strength and pH was not only to investigate the effects on surface activity of SKF 525-A HCl and chlorpromazine HCl, but also to correlate the relative hemolysis at these milliosmolar NaCl solutions.

Determination of the mean cellular volume of the red cells. The mean cellular volume of the red cells incubated under varying conditions was measured as follows: at the end of a 60-min incubation at 23°, 0·5 ml of red cell suspension was pipetted into a Hopkins vaccine tube and centrifuged at 2000 *g* for 20 min in an International centrifuge model K with horizontal head. After centrifugation, the hematocrit was recorded and the hemoglobin determined. The mean cellular volume of the remainder of intact red cells was calculated as a fractional value of the mean cellular volume in 300 mOsmole NaCl according to the equation: $V/V_o = h/h_o\ (1—p)$, where V is mean cellular volume of cells in varying milliosmolar NaCl; V_o is the mean cellular volume of cells in 300 mOmoles NaCl; h and h_o are representative hematocrits; p is a fraction of the hemolyzed cells.[15]

Determination of SKF 525-A uptake by red cells suspended in 300 mOsmole NaCl. The uptake of SKF 525-A by the red cells was studied spectrophotometrically. The u.v. absorption spectra of SKF 525-A HCl in chloroform with a Beckman DB recording spectrophotometer indicated that the E_{max} was at 257 mμ and followed Beer's Law. SKF 525-A HCl uptake experiments were identical to other experiments except that the volume ratio was increased 10 times. One ml of red cells from the stock red cell suspension was pipetted into a control tube (15 ml graduated conical centrifuge tube) containing 10 ml of 300 mOsmole NaCl. An identical amount of red cell suspension was pipetted into a tube containing 10^{-4} M SKF 525-A HCl in an identical volume of 300 mOsmole NaCl. The centrifuge tubes stoppered with parafilm were mixed gently by repeated inversions, incubated at 23° for 5 min and then centrifuged at 900 *g* with an International centrifuge, PR-2 model, for 20 min. An aliquot of the resultant supernatant was placed in 50-ml centrifuge tubes. SKF 525-A HCl (10^{-4} M) in control supernatant was prepared and served as a standard. The extinctions of both the supernatant from control red cells in the absence of SKF

525-A and the supernatant from red cells with the drug were extracted with 6 ml chloroform. The pH was adjusted to 9·0 by adding identical amounts of 1 N NaOH. SKF 525-A HCl was then extracted with 6 ml chloroform after shaking for 2 hr with an International extraction shaker. The extinctions of these samples were determined against a chloroform blank at 257 mμ with a Zeiss PMQ II spectrophotometer. The resultant extinctions were compared and the per cent SKF 525-A HCl uptake by the red cells was then calculated.

RESULTS

Effect of varying concentrations of SKF 525-A on the red cells suspended in varying milliosmols of NaCl. In preliminary experiments, it was demonstrated that the addition of SKF 525-A to the red cell suspension did not alter the typical sigmoidal osmotic hemolysis curve of red cells (Fig. 1). It can be seen that SKF 525-A can cause

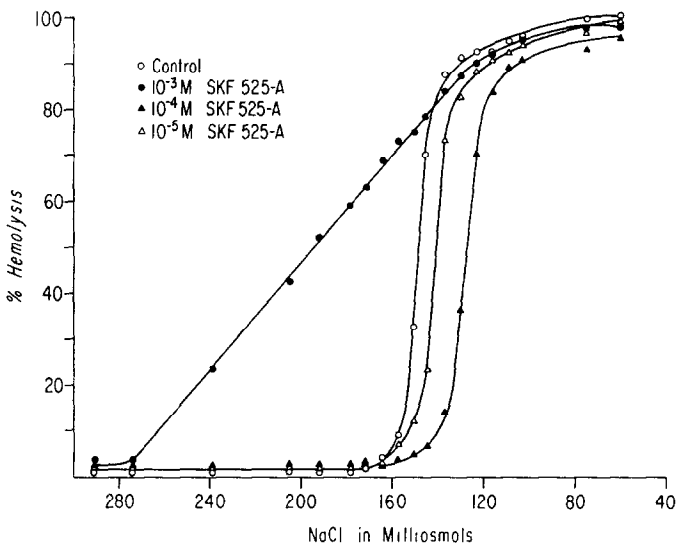

FIG. 1. Effects of SKF 525-A on osmotic hemolysis of human red blood cells obtained from 5 different donors were studied. The stock red cells were suspended in varying milliosmolar concentrations and incubated for 30 min at 23°. Each point on the graph represents the mean value of blood from 5 donors.

either hemolysis or stabilization of the red cell membrane. These effects are concentration dependent. Fifty per cent hemolysis of control red cells and of the red cells incubated with 10^{-5}, 10^{-4} and 10^{-3}M concentrations of SKF 525-A occurred at 148, 141, 127 and 194 mOsmole of NaCl respectively. A stabilization of the red cell membrane occurred at 10^{-4} and 10^{-5}M SKF 525-A, which is indicated by a shifting of 21 and 7 milliosmols from the control.

The degree of stabilization of the red cell membrane was determined. Red cells that were more osmotically resistant than the control red cells were able to tolerate increases of about 0·17 and 0·48 atmosphere pressure at 23° respectively. In contrast, at 10^{-3}M, SKF 525-A did not stabilize the red cell membrane but caused an extreme hemolysis and decreased the osmotic resistance by about 1·1 atmosphere pressure. It is interesting to note that the hemolysis of cells treated with 10^{-3}M SKF 525-A did

not show the typical "all-or-none" curve but rather a gradual hemolysis which occurred between 290 and 60 mOsmole.

Effects of temperature and incubation time on hemolysis and stabilization. The hemolysis of the red cells was temperature dependent (Fig. 2, bottom). The rate of red cell hemolysis increased as temperature was decreased. The rate of hemolysis at all

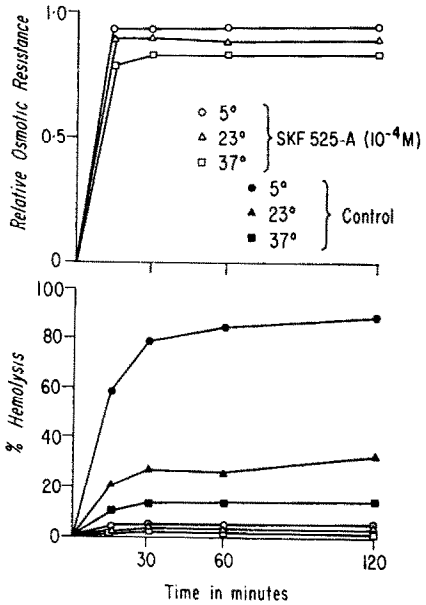

FIG. 2. Effects of temperature and incubation time on the hemolysis of control red cells and SKF 525-A-treated red cells were studied. The red cells were suspended in 150 milliosmolar NaCl and incubated at 5°, 23° and 37° for 15, 30, 60 and 120 min. Each point represents 5 different donors. Top. The relative degree of osmotic resistance was expressed from the following equation:

$$1 - \left[\frac{\%\text{ hemolysis (drug)}}{\%\text{hemolysis (control)}}\right].$$

Bottom. Time course of hemolysis of control cells and cells treated with SKF 525-A (10^{-4}M) at different temperatures.

temperatures occurred rapidly up to the first 30 min, and slowed thereafter up to 120 min. During the first 30 min of incubation, the average rate of hemolysis of the control red cells was 2·60 per cent min^{-1} at 5°, 0·90 per cent min^{-1} at 23° and 0·47 per cent min^{-1} at 37°, the comparable values of cells treated with SKF 525-A (10^{-4}M) were 0·13 per cent min^{-1} at 5°, 0·10 per cent min^{-1} at 23° and 0·07 per cent min^{-1} at 37°. The maximum reduction of the hemolysis rate was 20-fold at 5°. Nine- and 7-fold reductions were seen at 23° and 37°. The degree of red cell stabilization induced by SKF 525-A (10^{-4}M) was also expressed in terms of the relative osmotic resistance (Fig. 2, top). It appeared that the effect of SKF 525-A on the stabilization of the red cell membrane was immediate within the first 15 min of incubation, irrespective of temperature. After 15 min of incubation, a maximal stabilization of the red cell membrane was obtained. This stabilization effect was prolonged and could be demonstrated after 2 hr. The stabilizing effect of SKF 525-A on the red cell membrane was most effective at 5° and less at 23° and 37°. The extent of SKF 525-A induced

stabilization could not be estimated at 23° and 37° because of a very low degree of hemolysis of red cells at these temperatures as compared with that at 5°.

Surface tension measurements of SKF 525-A and chlorpromazine. Surface tension measurements of SKF 525-A and chlorpromazine at varying drug concentrations at different ionic strength and pH were determined. Varying concentrations of both SKF 525-A and chlorpromazine, identical to those used for osmotic hemolysis studies, were used for surface tension measurements.

It has been reported by a number of investigators[16–18] that chlorpromazine stabilizes the red cell membrane. It was postulated that this stabilization was due to its surface activity and adsorption of molecules on the red cell membrane.

From a structural standpoint, SKF 525-A has both nonpolar and polar groups, as does chlorpromazine. Both drugs are also highly lipid soluble. Therefore, surface activity and lipid solubility were investigated as possible factors responsible for the red cell membrane stabilization effect of SKF 525-A. These data indicated that the

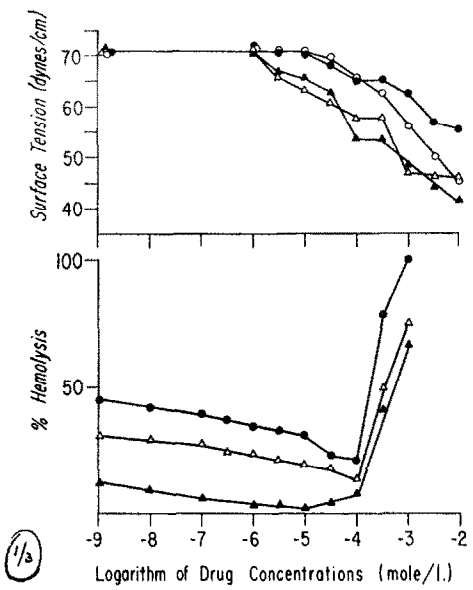

FIG. 3. Top. Comparative surface tensions of SKF 525-A and chlorpromazine were measured: △ = SKF 525-A in NaCl (ionic strength, 0·075; pH 5·30); ▲ = SKF 525-A in NaCl (ionic strength, 0·150; pH 5·70); ○ = SKF 525-A in NaCl (ionic strength, 0·075; pH 2·00); ● = chlorpromazine in NaCl (ionic strength, 0·075; pH 5·30). All of the surface tensions were measured at 23° and are expressed as dynes/cm.

Bottom. Comparative per cent hemolysis of red cell suspensions in 150 mOsmole NaCl with ● = chlorpromazine, △ = SKF 525-A (pH 5·30) and ▲ = SKF 525-A in 150 milliosmolar NaCl–PO_4 (pH 7·40) is plotted against varying concentrations of these compounds. An identical sample of red cells was used throughout to determine the comparative effects of SKF 525-A and chlorpromazine on the membrane stabilization.

maximum red cell membrane stabilization occurred at 10^{-4}M concentration of both SKF 525-A and chlorpromazine (Fig. 3, bottom). At this concentration, both SKF 525-A and chlorpromazine showed the first breaking points during measurements of surface tension which were 57·5 and 66·0 dynes/cm (Fig. 3, top). Thus, formation of micelles appeared to occur at this concentration. Furthermore, these surface activities

appeared to be correlated with the degree of membrane stabilization. The surface tension of SKF 525-A was 8·5 dynes/cm lower than that of chlorpromazine, and SKF 525-A stabilized the red cell membrane 15–20 per cent more than chlorpromazine. The surface activities of SKF 525-A appeared to be pH dependent. At higher pH (5·7) SKF 525-A demonstrated greater activity than at lower pH (2·00). At 10^{-4}M, SKF 525-A showed 53·5 dynes/cm at pH 5·70 v.s. 65·5 dynes/cm at pH 2·00. The membrane-stabilizing effects of both SKF 525-A and chlorpromazine were biphasic. Higher concentrations (10^{-3}M) of both SKF 525-A and chlorpromazine resulted in increased hemolysis. Their membrane-stabilizing effects were reversed and hemolysis was seen at this higher concentration. About 99 per cent hemolysis occurred with 10^{-3}M chlorpromazine, whereas 65 and 75 per cent hemolysis occurred with 10^{-3}M SKF 525-A at pH 5·30 and 7·40 respectively. In contrast, at dilute concentrations, both compounds maintained their stabilizing effects; SKF 525-A was capable of membrane stabilization at concentrations as low as 10^{-9}M.

SKF 525-A uptake by red cells. SKF 525-A experiments were carried out as described in the experimental Methods. Since it had already been determined that stabilization of the red cell membrane occurred at 10^{-4}M SKF 525-A, it was of interest to determine how many molecules of SKF 525-A were taken up by the red cells. The red cells from four different donors were incubated with 10^{-4}M SKF 525-A for 5 min at 23°. The concentration of SKF 525-A in the supernatant was extracted with chloroform and quantitated spectrophotometrically. The number of SKF 525-A molecules adsorbed by a single red cell was estimated to be approximately $3{\cdot}2 \times 10^8$ molecules at 10^{-4}M SKF 525-A. If the mean red cell surface area is assumed to be 163 square microns, it can be calculated that 1 molecule would occupy 51 Å^2 of red cell area. These data coincided remarkably well with the number of chlorpromazine molecules adsorbed per single red cell at 10^{-4}M concentration of chlorpromazine.[18] Furthermore, at pH 7·4, SKF 525-A stabilized the red cell membrane more effectively than at lower pH, e.g. pH 5·3.

TABLE. 1. THE RELATIVE DEGREE OF OSMOTIC RESISTANCE OF HUMAN RED CELLS TREATED WITH SKF 525-A AND CHLORPROMAZINE IN VARIOUS 150 MOSMOLE SALT SOLUTIONS AND AT DIFFERENT PH*

Treatment	KCl (pH 5·30)	NaCl (pH 5·30)	NaCl–PO_4 (pH 7·40)
Chlorpromazine HCl (1×10^{-4} M)	0·62 ± 0·03	0·70 ± 0·04	0·80 ± 0·03
SKF 525-A HCl (1×10^{-4} M)	0·84 ± 0·05	0·87 ± 0·06	0·92 ± 0·06

* Relative degree of osmotic resistance was calculated as follows:

$$1 - \left[\frac{\%\text{ hemolysis (drug)}}{\%\text{ hemolysis of control}}\right]$$

. Figures represent means ± S.D. of 4 individual experiments.

Effects of salt and pH on the relative degree of osmotic resistance of human red cell treated with 10^{-4}M SKF 525-A or chlorpromazine. The relative degree of osmotic resistance was calculated according to the equations shown in Table 1. The degree of osmotic resistance of the red cell was significantly increased by both SKF 525-A and chlorpromazine, irrespective of the pH or salt solutions studied. However, the optimal membrane stabilization was obtained with an NaCl–PO_4 (pH 7·40) test solution. High

standard deviations were due to variations of hemolysis found in normal blood samples. SKF 525-A increased the osmotic resistance of the red cells by 20 per cent more than chlorpromazine at the same concentration. The red cells incubated with 150 milliosmolar (pH 5·30) NaCl and NaCl–PO_4 (pH 7·40) showed a slight increase in the osmotic resistance of red cells over the resistance of red cells incubated with an identical mOsmole KCl (pH 5·30) solution.

Effects of KCl and NaCl on red cell osmotic hemolysis. It was observed from the preceding experiments that KCl caused greater hemolysis than equimilliosmolar NaCl. Therefore the comparative effects on identical red cells in varying milliosmolar concentrations of KCl and NaCl were studied. From the osmotic hemolysis curve (Fig. 4) it was evident that KCl resulted in a shift of approximately 7 mOsmole 50 per cent hemolysis. These experiments were repeated with samples from four different donors and the same results were obtained.

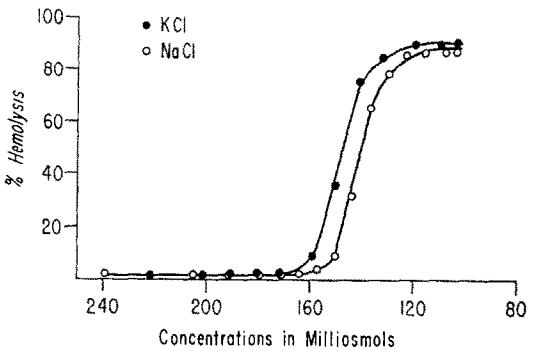

FIG. 4. Identical red cells from one healthy donor were incubated with varying milliosmolar concentrations of either KCl or NaCl at 23° for 60 min. At the end of incubation, the per cent hemolysis was plotted. Each point represents the mean value of duplicate samples from a single donor.

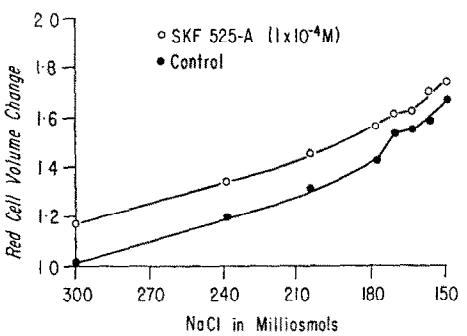

FIG. 5. Identical red cells were incubated in duplicate at varying milliosmolar concentrations of NaCl with or without SKF 525-A (10^{-4}M) at 23° for 60 min. Red cell volume change was calculated from the equation $V/V_o = h/h_o$ (1—p) where h_o is the initial hematocrit of control (300 mOsmole NaCl); V_o is the mean cellular volume of control cells (300 mOmole NaCl); h is the hematocrit of samples; (1—p) is the fraction of intact cells of the samples.

Effects of SKF 525-A on human red cell volume. Determinations of mean cellular volume were performed as described in Methods. These data showed that the presence of SKF 525-A (10^{-4}M) caused a change in the mean cellular volume of the red cells over all concentration ranges studied (Fig. 5). Red cells treated with SKF 525-A

(10^{-4}M) showed a 16 per cent increase in the mean cellular volume at the 300 mOsmole NaCl concentration as compared to control red cells at the same milliosmolar concentration. The mean cellular volume of SKF 525-A-treated cells continued to increase between 7 and 15 per cent as compared to control red cells. It appeared that in the presence of SKF 525-A the mean cellular volume increased further than control, with hemolysis starting at a lower concentration as compared to the control red cells.

DISCUSSION

The stabilization of biological membranes by pharmacologically active tranquilizers, antihistamines, barbiturates, steroids and local anesthetics is well known.[19, 20] All of these drugs exert a biphasic effect on biological membranes which is drug concentration dependent. The most common physicochemical properties of these drugs are associated with their surface activity and high lipid solubilities at physiological pH.

Recent reports have indicated that chlorpromazine and other phenothiazine derivatives accumulate at the biological membranes.[16–19] The degree of accumulation of a drug at a membrane depends on its surface activity. The size of micelles has been postulated as a reason for differences in pharmacological action.[21] However, the capacity for molecular interactions and the structural affinities at the membrane site would contribute equally to the pharmacological action of the drug.

It is evident from the data presented here that SKF 525-A at low concentrations protects the red cell membrane against hypotonic NaCl solutions. However, at higher concentrations, SKF 525-A causes hemolysis of red cells to occur even at near isotonic NaCl concentrations. Since membrane stabilization is still measurable at concentrations as low as 10^{-9}M, SKF 525-A appears to be highly potent. The effect of SKF 525-A on membrane stabilization is also instantaneous and prolonged. It should be noted that this phenomenon appears to correlate well with activity *in vivo*. The inhibitory action of SKF 525-A on the microsomal enzyme systems *in vivo* is rapid and prolonged and appears nearly maximal at 10^{-4}M *in vitro* and *in vivo*.[9]

The degree of red cell membrane stabilization appears to correlate with the degree of surface activity of SKF 525-A, which is comparable to the surface activity of chlorpromazine. Furthermore, at higher pH the degree of membrane stabilization is increased. This increase in stabilization may be associated with the increased formation of undissociated molecules or undissociated molecular aggregates of SKF 525-A.

Membrane stabilization induced by SKF 525-A does not appear to be attributed to an inhibition of transmembrane water transport. The mean cellular volume after SKF 525-A (Fig. 5) is significantly increased over a wide range of varying salt concentrations when compared to control mean cellular volumes at the same salt concentration. The increase in the mean cellular volume of 7–15 per cent found experimentally may be sufficient to account for the membrane stabilization phenomenon. Thus, SKF 525-A permits the red cell to achieve a larger critical volume than control, and therefore may account in part for the stabilizing effect. Other mechanisms of the membrane stabilization can be suggested. Biological membranes under osmotic pressure equal to 0·5 atmosphere pressure must require a tremendous level of energy to maintain membrane integrity. The membrane penetration of SKF 525-A may not only support the membrane structure, but may also possibly expand the membrane surface area as well.

Possible hydrophobic molecular interactions between SKF 525-A and the biological membrane may not be limited to the nonpolar groups of lipids but may extend to nonpolar moieties of protein as well. The number of SKF 525-A molecules adsorbed per single red cell, at the concentration at which maximum stabilization of the membrane occurs, is calculated to be equivalent to one molecule per 51 Å^2 red cell area. It is interesting to note that this figure agrees well with that calculated for promethazine.[19] The maximal promethazine stabilization of the red cell membrane requires one molecule per 65 Å^2 area of the red cell. The number of molecules adsorbed by a single red cell is calculated under the assumption that SKF 525-A is adsorbed by the membrane. The membrane penetration and intracellular concentration of SKF 525-A must be determined before more accurate figures can be derived. This hypothesis is supported by other studies,[22–24] such as studies of the monomolecular film at air–water interfaces. The extent of penetration and strength of soluble surfactants into insoluble films depends on many factors such as chemical nature, number of polar groups in two molecular species, van der Waals forces between nonpolar groups, surface area of the insoluble film, concentration of the dissolved compounds, pH and ionic strength of the solutions and the steriospecificity of two molecular species. Skou[25] and Shanes[26] have also suggested that the intramembrane accumulation of various membrane stabilizers resulted in an increased membrane pressure with a consequent expansion of the membrane.

The effect of SKF 525-A on the red cell metabolism cannot be ruled out entirely. However, membrane stabilization by SKF 525-A via altered red cell metabolism seems unlikely under the experimental conditions to which the red cells were subjected.

Considering both the multi-inhibitory action of SKF 525-A on the intact liver microsomal enzymes and its physicochemical properties, it is tempting to postulate that these diverse inhibitory actions may be attributed to a membrane phenomenon. However, SKF 525-A does competitively inhibit *N*-deethylation, according to Anders and Mannering,[11] which may be in addition to a membrane effect since SKF 525-A has the diethylamine substitution.

Hollunger[10] has also suggested that SKF 525-A acts as a competitive inhibitor for the microsomal amidase enzyme. It appears that the mode of SKF 525-A inhibition on the microsomal enzymes may involve both effects. To resolve this, further kinetic studies with soluble microsomal enzyme preparations are necessary.

It should be noted (Fig. 4) that in contrast to a prior report,[27] KCl causes more hemolysis than NaCl; at 50 % hemolysis, KCl demonstrates a shift of 7 mOsmole, which is equivalent to 0·16 atmosphere pressure. Why potassium ions cause more hemolysis than sodium ions at equimolar concentrations is an intriguing question. In many experiments potassium ions have been widely used as a substitute for sodium ions. At present, it appears that potassium ions may have a direct membrane effect. Further studies are in progress and will be reported in a subsequent paper.

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