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EFFECT OF SULOCTIDIL [1(4-ISOPROPYLTHIOPHENYL)-2-*n*-OCTYLAMINOPROPANOL] ON HUMAN PLATELETS *IN VITRO**

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Abstract—Platelets exposed to high concentrations of suloctidil [1(4 isopropylthiophenyl)-2-*n*-octylamino-propanol] in plasma or suspending fluid were sphered and swollen like a blown-up balloon, giving rise to the term "ballooned platelets." All of the subcellular organelles were altered drastically except for the granules which were well preserved and often concentrated around the periphery at one side of the plasma membrane, especially in platelet-rich plasma. Platelets treated with chlorpromazine showed more severely damaged plasma membrane which seemed unable to retain the well preserved granules. The frequency of platelets showing these distinctive morphological changes increased with increasing concentrations of suloctidil and correlated with percentage loss of [^{51}Cr]sodium chromate which is a cytoplasmic marker. Washed human blood platelets slowly lose their store of preabsorbed serotonin when incubated at 37° with suloctidil in a concentration between 0.06 and 6 μM. No simultaneous loss of other stored compounds (low affinity platelet factor 4 antigen or adenine nucleotides) was observed. At higher concentrations of suloctidil (20–60 μM in the absence of soluble proteins), a fast loss of cytoplasmic material was observed while the contents of the storage granules were partially retained. High suloctidil concentrations caused a drop in metabolic ATP, accumulation of IMP and loss of conversion to inosine and hypoxanthine. By addition of soluble protein to the incubation mixture or performance of the experiments in plasma medium, the necessary concentrations of suloctidil to obtain a similar effect had to be increased by a factor of 10. Suloctidil at a 6 μM concentration exerted a membrane-stabilizing effect on human erythrocytes while 20 μM caused increased and 60 μM complete hemolysis. The drug thus interferes with the stability of the plasma membrane in a biphasic way: it stabilizes at a low concentration and disrupts at a higher concentration so that it can be characterized as a surface-active agent and it causes a slow specific loss of biogenic amines from storage granules while increasing the retention of other stored compounds

It was suggested recently that suloctidil [1(4-isopropyl-thiophenyl)-2-*n*-octylaminopropanol], a vasodilating drug, can also act as an antithrombotic agent by interfering with platelet functions. De Gaetano *et al.* [1] reported that exposure of human platelets to suloctidil *in vitro* partially inhibited collagen-induced aggregation. By contrast, Mills and Macfarlane [2] found that suloctidil in pharmacological doses *in vitro* did not inhibit ADP-induced platelet aggregation and malondialdehyde production of human platelets in plasma. However, they did report that suloctidil caused a progressive loss of preabsorbed serotonin from the platelets over 2–4 hr, that a half-maximal effect occurred with 8.3 μM suloctidil and that higher concentrations caused inhibition of serotonin uptake. Results similar to the latter were reported for platelet-rich rat plasma [3]. The present study was undertaken to investigate the effects of a wide range of concentrations of suloctidil on washed platelets as well as on platelets in plasma (PRP). The ultrastructure and the biochemistry of platelets preincubated with [^{3}H]serotonin and [^{14}C]adenine, and the release of low affinity platelet factor 4 (LA-PF$_4$) antigen were studied.

*A preliminary presentation of some of these data was made to the 61st Annual FASEB Meeting, and an abstract printed in *Fed. Proc.* **36**, 1082 (1977).

MATERIALS AND METHODS

Suloctidil (mol. wt 337) was supplied by Continental Pharma, Brussels, Belgium, and was dissolved in acetone and diluted with either acetone or ethanol.

Radiochemicals were [^{51}Cr]sodium chromate (500 Ci/g) from Mallinckrodt Nuclear, Inc., St. Louis, MO, [^{3}H]serotonin-binoxalate (24.9 Ci/m mole) from New England Nuclear, Boston, MA, and [U-^{14}C]adenine (286 Ci/mole) from Amersham/Searle Corp., Chicago, IL. 2,5-Diphenyloxazole (PPO) and bis [2(5-phenyloxazolyl) benzene] (POPOP) were from Sigma Chemical Co., St. Louis, MO. Imipramine was a gift from the Ciba–Geigy Corp., London, U.K. and Summit, NJ. Thrombin was provided by Parke-Davis, Detroit, MI. Purified chlorpromazine HCl was a gift from Dr. Martin W. Adler, Department of Pharmacology, Temple University Health Sciences Center.

Three types of platelet preparations were tested for their response to suloctidil.

Method 1: freshly drawn human blood anticoagulated by acid citrate dextrose (ACD) was obtained from the local blood bank and used for preparation of the platelet-rich plasma which was used after adjustment of pH to 7.6.

Method 2: human platelets from ACD blood were washed by the method of Mustard *et al.* [4]. Final suspension of platelets was made in Tyrode's solution to which was added 0.3% bovine serum albumin and 1

unit potato apyrase/ml. In some experiments, platelets, after two washings, were resuspended in platelet-poor plasma (PPP) containing 0.5 units apyrase and 5 units heparin/ml. In these experiments, platelets suspended in Tyrode albumin solution containing the same amount of heparin and apyrase were used as controls for the effects of these agents.

Method 3: blood was collected in the laboratory from healthy donors for the preparation of saline-washed protein-free platelet suspensions. In one case, blood was drawn into 0.1 vol. of 0.11 M Na-citrate, pH 7.8–7.9. PRP was prepared and adjusted to pH 6.0 by the addition of 0.11 M citric acid. The platelets were washed twice with 0.15 M NaCl containing 11 mM Na-citrate, pH 6.0. The platelets were resuspended in 0.15 M NaCl at pH 6 [5]. In another case, blood was drawn into 0.07 vol. of 0.077 M EDTA, pH 7.4. The platelets were separated and washed twice with saline containing 0.1 mM EDTA, pH 7.4 [6].

The platelet-rich plasma prepared by 15-min centrifugation at 250 *g* and room temperature was incubated with ^{3}H-labeled serotonin-binoxalate (1 μCi/ml) and ^{14}C-labeled adenine (250 nCi/ml) by shaking for 20 min at 37° in a Dubnoff metabolic incubator (75 strokes/min). This took place before pH adjustment for the citrated plasma. No difference was observed between preparations using citrate or EDTA as anticoagulant in protein-free suspending medium.

Electron microscope studies were performed with platelets in plasma and platelets washed by method 3. Suloctidil was added in 10 μl acetone/ml of plasma or in a 1.25 μl ethanol–acetone mixture (1:13)/ml of platelet suspension. Platelets incubated with the same concentration of acetone served as controls. The platelets were fixed initially in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at room temperature for 30 min followed by cold fixation for 1–2 hr. The platelets were then fixed in cold 1% osmium tetraoxide for 30 min before washing and dehydration with ethanol and embedding in Epon 812. Sections were stained with uranyl acetate followed by lead citrate.

Loss of ^{51}Cr from platelets in plasma and platelets washed by the method of Mustard *et al.* [4] (method 2) was compared. [^{51}Cr]sodium chromate (100 μCi/ml) was added to the suspension of platelets after the first washing (0.2–0.5 ml ^{51}Cr/2 × 10^{10} cells). The excess of label was removed by the subsequent washings. Loss of ^{51}Cr radioactivity from the platelets was determined in the supernatant fluid after sedimentation of platelets in an Eppendorff centrifuge (2 min, 7500 *g*).

Suspensions of platelets were examined by phase contrast microscopy. The number of ballooned platelets was counted in a Neuebauer chamber.

The studies correlating loss of cytoplasmic nucleotides with liberation of serotonin and low affinity platelet factor 4 (LA-PF_4) antigen [7] were performed in washed platelets prepared by the citrate or EDTA method (method 3). Imipramine (2.5 μM) was present during incubation to prevent re-uptake of serotonin [8]. Tritium was determined in the total incubate after stopping the experiment by cooling in an ice bath and in the supernatant fluid after centrifugation for 20 min at 900 *g*. Samples of 25 μl were added directly to a scintillation mixture consisting of toluene with a PPO and POPOP [5] mixture with an equal volume of Triton X-100. The counts in the ^{3}H-channel were corrected for spill over from ^{14}C. [^{14}C]adenine metabolites were determined in the local incubate and supernatant fluid by paper strip electrophoresis, according to the method of Holmsen and Weiss [9], and counted in toluene with PPO and POPOP [5]. All radioactive determinations were done in a Beckman LS330 scintillation counter. Adenylate energy charge (AEC) was determined by the ratio between the ^{14}C-labeled platelet ATP, ADP and AMP according to the formula first proposed by Atkinson [10] and adapted to use in cells with two adenine nucleotide compartments by Mills [11]. Low affinity platelet factor 4 (LA-PF_4) antigen was determined by radial immunodiffusion as described by Niewiarowski *et al.* [7].

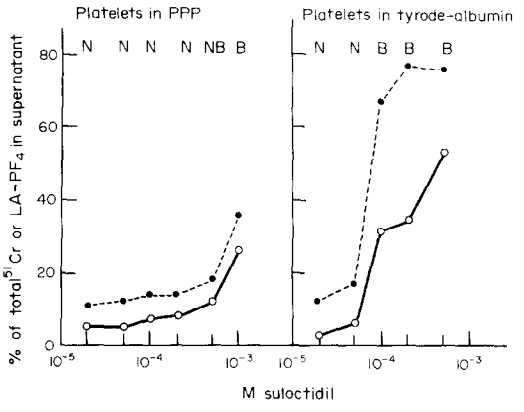

Fig. 1. Correlation between loss of ^{51}Cr and low affinity platelet factor 4 antigen and ballooning of platelets. The platelets were prepared by the method of Mustard *et al.* [4] and incubated in Tyrode's solution for 1 hr at room temperature. Low affinity platelet factor 4 antigen [7] and ^{51}Cr in the platelet supernatant fluid were determined as per cent of the content in the whole platelet suspension. N = normal platelets; B = ballooned platelets (by phase microscopy). Key: (●– – –●). ^{51}Cr, and (○——○), low affinity platelet factor 4 in supernatant fluid.

RESULTS

Exposure of platelets in plasma or in suspending fluid to suloctidil (1 mM and 60 μM, respectively) resulted in striking morphological changes that were visible even at the level of light microscopy. The platelets were sphered and swollen like a blown-up balloon giving rise to the term "ballooned platelets." The percentage of ballooned platelets in a preparation increased with increasing concentration of the drug. PPP inhibited the loss of ^{51}Cr radioactivity and of LA-PF_4 antigen from platelets and prevented ballooning (Fig. 1). The inhibition was overcome by a 10- to 20-fold increase in drug concentration. Aggregation induced by collagen was lost when 100 per cent of the platelets were ballooned. At lower suloctidil concentrations (12 μM in suspending fluid) the degree of ballooning and loss of aggregation was comparable.

At the ultrastructural level, ballooned platelets had a unique appearance. All of the subcellular organelles were altered drastically except for the granules which were very well preserved and often concentrated around the periphery at one side of the plasma membrane, especially in platelets which were suspended in plasma.

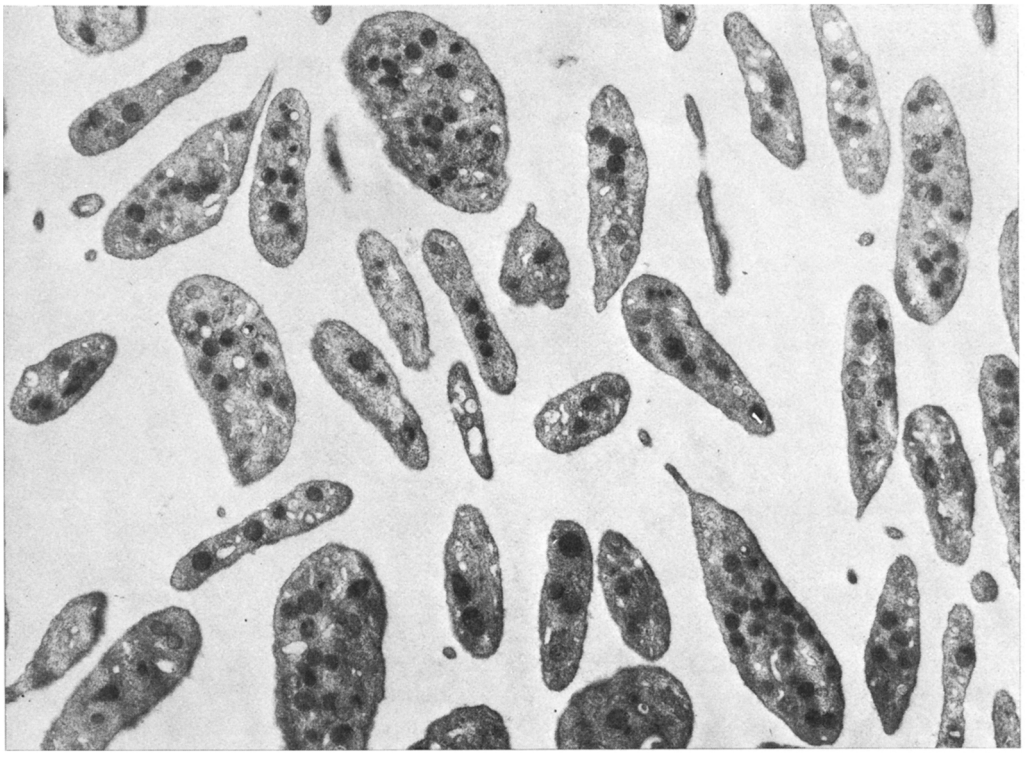

Fig. 2. Transmission electron micrograph of human platelets in PRP. An amount of acetone (10 μl) equal to that serving as carrier for suloctidil was added to the PRP. The platelets were typical of normal platelets with few pseudopods. Of importance to this study, the granules were randomly distributed and the cytoplasm was of typical electron density and finely granular. × 6,800.

The cytoplasm had become flocculent and in many cases quite sparse. Occasional small breaks were found in the plasma membrane of suloctidil-treated platelets but never in control platelets that were processed in parallel with the treated platelets (Figs. 2–5). Platelets treated with chlorpromazine showed a more severely damaged plasma membrane which seemed unable to retain the well preserved granules (Fig. 6).

Since [^{14}C]adenine is incorporated into the metabolic (cytoplasmic) adenine nucleotides and since nucleotides cannot penetrate the intact platelet membrane by diffusion, the loss of ^{14}C-nucleotide radioactivity from platelets was used as an index for the loss of cytoplasmic components. Suspensions of platelets washed by method 3 showed an excessive loss of intracellular material when exposed to 30–60 μM suloctidil. This loss varied for the different intracellular constituents. For instance, at 60 μM suloctidil there was 100 per cent loss of cytoplasmic nucleotides (indicating free exchange between internal and external space), while only 76 per cent of serotonin label and 47 per cent of LA-PF$_4$ were recovered extracellularly (Fig. 7). By contrast, thrombin released about 100 per cent of the stored serotonin and at least as much of the LA-PF$_4$ antigen, while loss of cytoplasmic nucleotides was only slightly higher than what was obtained in the controls (with no additions).

The levels of ^{14}C-labeled adenine metabolites changed with increasing suloctidil concentrations in a manner which correlated with increased loss of cell constituents and cell destruction. The [^{14}C]ATP level and adenylate energy charge decreased while ADP, AMP and IMP showed increased accumulation, most expressed in IMP. An increase in inosine and hypoxanthine accompanied a moderate increase in leakage, while the complete destruction of cell organization was accompanied by a drop in inosine and hypoxanthine (Table 1). The values contrasted with those obtained with thrombin which caused secretion of stored compounds but only very limited loss of cytoplasmic constituents and a moderate drop in metabolic ATP, little change in ADP and AMP and a lesser increase in IMP but a higher increase in inosine and hypoxanthine (Table 1 and Fig. 7).

A time-dependent, specific liberation of serotonin was seen at suloctidil concentrations between 0.06 and 6 μM (Fig. 8). This was not accompanied by leakage of cytoplasmic nucleotides (Fig. 9) nor by liberation or secretion of adenine nucleotides or LA-PF$_4$ antigen (after 1 hr with 6 μM suloctidil, 45 per cent release of [^{3}H]serotonin was accompanied by loss of 2 per cent LA-PF$_4$ antigen and less than 10 per cent of adenine nucleotides). With 20 μM suloctidil, considerably more extracellular serotonin was observed than with 6 μM with the difference being most marked after short-time incubation (Fig. 9).

Suloctidil showed a biphasic effect on human red cell fragility (Fig. 10). With three different dilutions of saline (0.080, 0.087 and 0.095 M NaCl), a statistically significant increase in resistance to hemolysis was observed when the suloctidil concentration was increased from 0 to 6 μM while higher concentrations caused a drastic increase in hemolysis.

Chlorpromazine HCl caused a parallel loss of cyto-

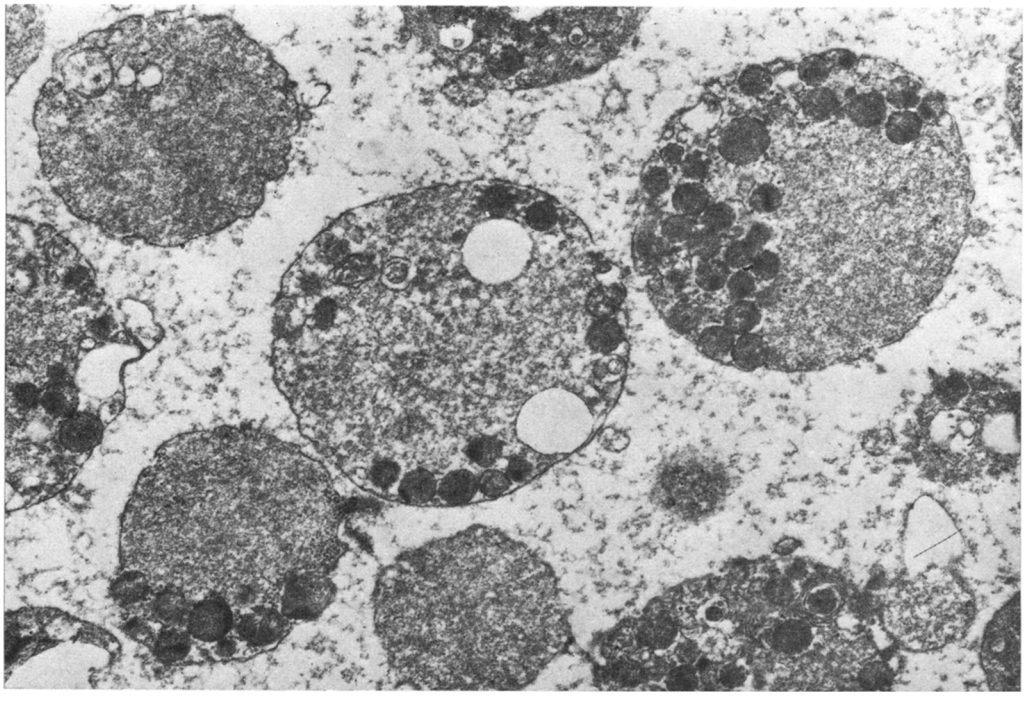

Fig. 3. Transmission electron micrograph of platelets (aliquot of same PRP as shown in Fig. 2) exposed to 1 mM suloctidil at 37° for 5 min before fixation. All of the platelets were sphered and had undergone extensive alteration of subcellular organelles. Mitochondria were no longer identifiable morphologically but myelin figures, possibly arising from mitochondria, were present. The large vacuoles were not typical of the surface-connecting system and the texture of the cytoplasm was changed. Only the granules were unchanged. These were usually clumped eccentrically at the periphery of the cell. The cytoplasm showed varying degrees of loss and alterations in density and texture. × 15,000.

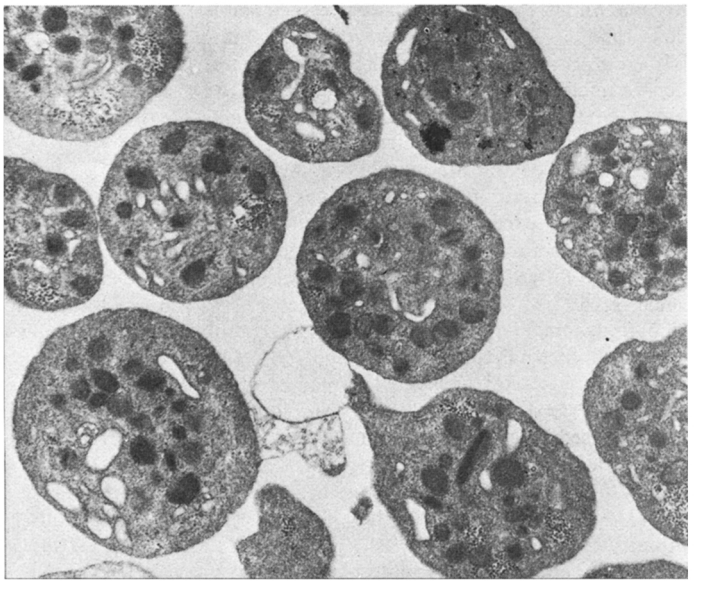

Fig. 4. Transmission electron micrograph of human platelets washed by method 3 and incubated for 5 min in buffered saline at 37°. × 12,300.

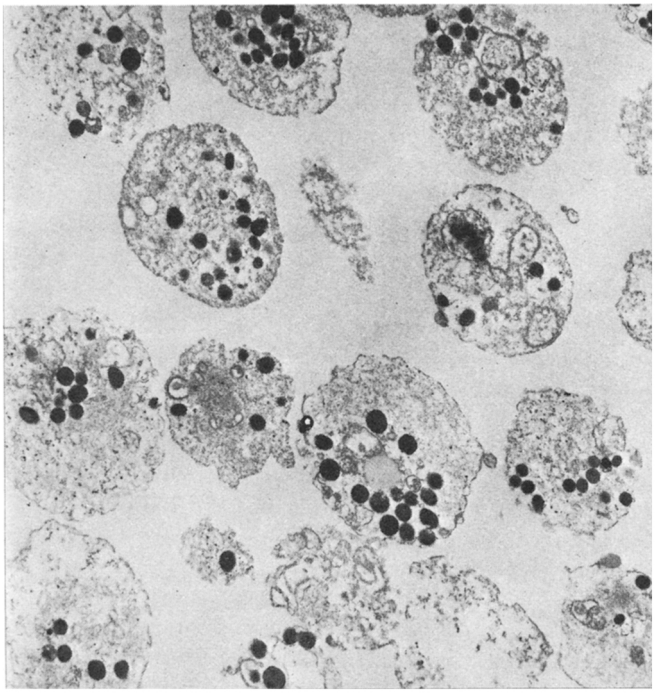

Fig. 5. Transmission electron micrograph of platelets washed by method 3 and exposed to 60 μM suloctidil for 5 min at 37°. The changes observed in Fig. 3 are evident in this preparation. Also, small breaks in the membrane were very common. × 7,800.

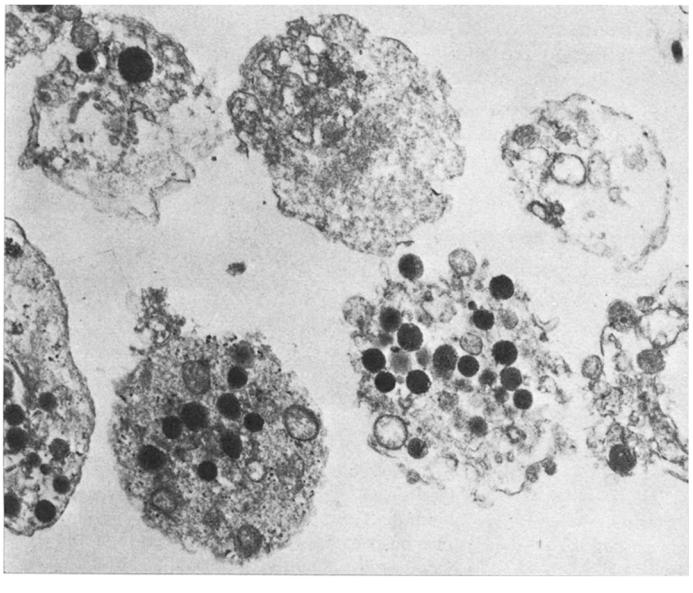

Fig. 6. Transmission electron micrograph of human platelets washed by method 3. The platelets were exposed to 300 μM chlorpromazine at 37° for 5 min before fixation. There was some variation in the extent of alteration from one platelet to another. The more severely affected ones showed extensive membrane alterations and loss of both granules and cytoplasm. × 12,900.

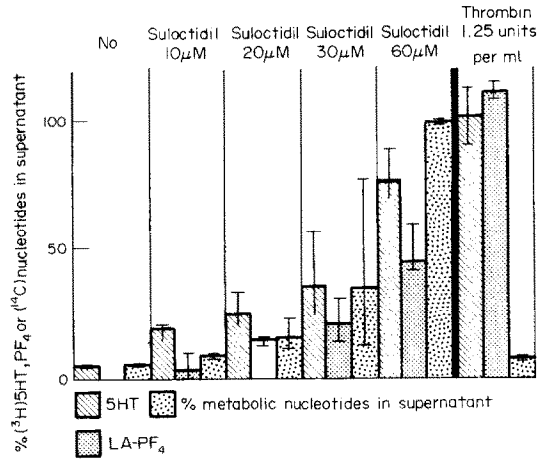

Fig. 7. Correlation between loss of preabsorbed [^{3}H]-5-hydroxytryptamine (5-HT or serotonin), low affinity platelet factor 4 antigen (LA-PF_4) and metabolic ^{14}C-labeled nucleotides. Experimental conditions are as described in Table 1. The platelet parameters were determined in the extracellular medium and in the whole platelets as described under Materials and Methods, and the amounts recovered extracellularly are given as per cent of total amount in the platelet incubate. Incubated by 10 min shaking at 37°. The range of results are given in the figure. Mean of three experiments.

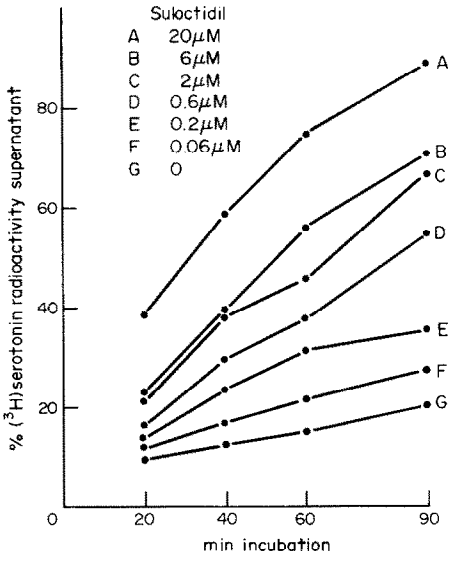

Fig. 8. Specific release of serotonin by low concentrations of suloctidil. Platelets were incubated at pH 7.6 in a medium containing 25 mM Tris–HCl of pH 7.4, 10 mM sodium citrate of pH 7.9, and 2.5 μM imipramine in saline. Suloctidil was added in 1.25 μl ethanol–acetone (6:1–1000:1)/ml of incubation medium. These amounts of organic solvents had no effect on platelet activities under the experimental conditions. Mean of two experiments.

plasmic marker and [^{3}H]serotonin from platelets. The loss of LA-PF_4 antigen was four times greater with 300 μM chlorpromazine than with 60 μM suloctidil, with the same degree of loss of cytoplasmic constituents. The drop in the level of [^{14}C]ATP in platelet suspensions was small and did not increase with increased loss of cytoplasmic material (Fig. 11).

DISCUSSION

Our experiments show that suloctidil had profound effects on the ultrastructure and biochemistry of human platelets *in vitro*. These effects were similar in the presence and absence of plasma proteins but, as might be expected, the concentration of drug required to produce changes was higher in the presence of protein. Part of the ultrastructural and biochemical changes apparently resulted from an increase in plasma membrane permeability that permitted escape of intracellular substances (even ^{51}Cr-labeled proteins) while retaining the storage granules. The loss of morphologically intact mitochondria and of serotonin from retained storage granules might result from direct action of the drug on mitochondrial and storage granule membranes

Table 1. Distribution of ATP metabolites after exposure to high concentrations of suloctidil*

	Distribution (%) of [^{14}C]ATP metabolites						
	Suloctidil (μM)						Thrombin (1.25 units/ml)
	0	10	20	30	(Range)	60	
ATP	76.1	71.9	63.9	51.0	(17.9–69.3)	12.0	49.9
ADP	10.7	10.2	11.9	12.5	(10.6–15.4)	29.2	9.4
AMP	1.9	2.6	3.5	6.7	(2.6–14.5)	12.3	3.5
IMP	1.8	3.8	6.8	15.6	(5.1–32.4)	39.1	11.3
Inosine + hypoxanthine	9.3	11.2	13.7	15.2	(11.6–19.4)	7.3	25.5
Adenylate energy charge	0.917	0.907	0.878	0.776	(0.535–0.899)	0.495	0.868

* Washed platelets prepared by method 3 with citrate had been prelabeled with [^{14}C]adenine and [^{3}H]serotonin. Four ml of the platelet suspension with 10 mM citrate of pH 7.9, 25 mM Tris–HCl of pH 7.4 (final pH 7.6), 0.13 M NaCl and 2.5 μM imipramine was incubated with varying concentrations of suloctidil added in 5 μl ethanol–acetone (1:1–6:1) or with 50 μl thrombin in saline. Incubation time was 10 min at 37°. Adenine metabolites were determined by paper electrophoresis as described in Materials and Methods. Adenylate energy charge was defined by the ratio between ^{14}C-labeled adenine nucleotides as follows $[ATP] + \frac{1}{2}[ADP]/[ATP] + [ADP] + [AMP]$. There was little variation between the experiments except at 30 μM suloctidil, where the range is given in parentheses. This seems to be the concentration where different sensitivities to suloctidil are indicated. Mean of three experiments.

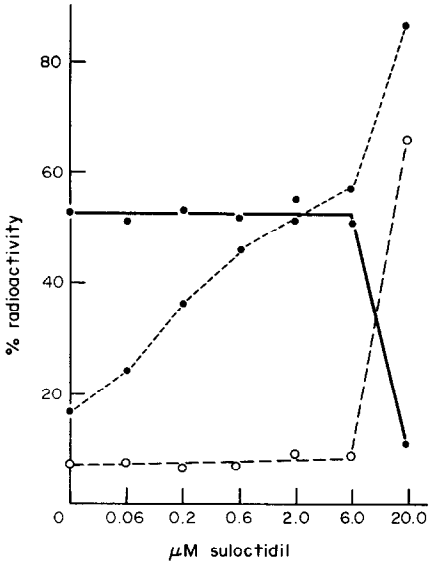

Fig. 9. Lack of correlation between specific release of serotonin, loss of cytoplasmic nucleotides and decrease in metabolic ATP level. Release of preabsorbed [^{3}H]serotonin, level of [^{14}C]ATP in whole platelet suspensions and loss of cytoplasmic ^{14}C-nucleotides to the extracellular medium were measured after 60 min of incubation at 37° and monitored as described in Materials and Methods and in Table 1. All numbers are in per cent of total ^{14}C-radioactivity (ATP, ●——●), total ^{14}C-nucleotide radioactivity (loss of cytoplasmic nucleotides, ○— — —○) or total ^{3}H-radioactivity (released serotonin, ● · · · ●) in the whole platelet incubate. Mean of two experiments.

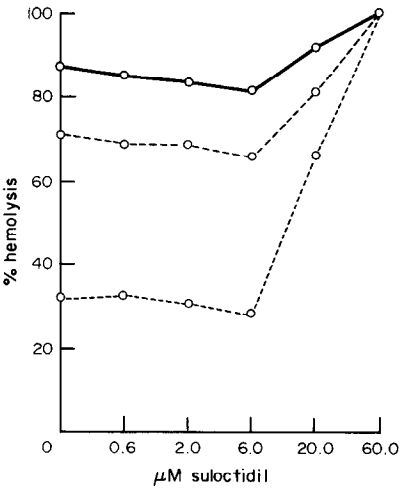

Fig. 10. Effect of suloctidil on red cell fragility. Experiments were performed by a modification of a general method for testing red cell fragility developed for Temple University Hospital. Isolated red cells from EDTA-anticoagulated blood were washed with 10 vol. saline which contained 10 mM Tris–HCl of pH 7.4. The washed cells were suspended in saline with 25 mM Tris–HCl of same pH to a final concentration of red cells of 1/50 that in whole blood. The suspension was incubated for 1 hr at 37° with 1.25 ml ethanol or ethanol:acetone containing increasing concentrations of suloctidil. From the incubation mixture were taken 0.2-ml samples which were added to 5 ml of an NaCl solution to a final concentration of 0.080, 0.087 and 0.095 M NaCl. The samples were refrigerated overnight and centrifuged. A spatula tip of sodium dithionite was added to the supernatant fluid and the spectrum measured immediately afterwards in a Beckman Acta III spectrophotometer by scanning from 450 to 350 nm. A reading was made of the peak of the curve. The hemolysis obtained with 60 μM suloctidil was taken as 100 per cent since in this case the degree of hemolysis was the same with all three hypotonic NaCl concentrations. NaCl concentrations were: ○—— ○, 0.095 M, ○— — —○, 0.087 M and ○ · · · ○, 0.080 M. The 0.095 M and 0.080 M curves represent the mean of four experiments and the 0.087 M curve the mean of two experiments. We established, by applying Student's *t*-test for paired numbers, that with 6 μM suloctidil the decrease in fragility as compared with no suloctidil addition was very significant, with $t = 6.27$ for ten sets of values and $P < 0.1$ per cent.

or they might be secondary to changes in the ionic content within the platelets.

Increased permeability of the plasma membrane was shown morphologically by the numerous small openings in the membrane which were especially prominent in the absence of added protein (Fig. 5). This was confirmed by the loss of labeled nucleotide metabolites (Table 1, Fig. 7) and the loss of the cytoplasmic marker ^{51}Cr from prelabeled platelets (Fig. 1.). The disappearance of microtubules might be secondary to increased permeability allowing alterations of the calcium concentration, thereby influencing microtubule assembly [12].

Suloctidil had a biphasic effect on blood platelets as indicated by the observations presented in Fig. 9. At concentrations of suloctidil up to 6 μM, there was no loss of nucleotides to the external medium (i.e. the nucleotide level remained at the low level of controls). However, at 20 μM suloctidil or above, nucleotides were released at a very rapid rate. Also, the previously stable level of metabolic ATP fell at a very rapid rate. While there had been a progressive loss of radioactivity from [^{3}H]serotonin-labeled platelets, the rate of loss increased sharply at 20 μM suloctidil. The response of erythrocytes to suloctidil was also biphasic (Fig. 10). These observations suggest that suloctidil is a membrane active drug stabilizing membranes at low concentrations and causing membrane disruption at high concentrations.

The loss of serotonin from platelets which retained adenine nucleotides and LA-PF$_4$ indicated that one of the results of the exposure of platelets to suloctidil was a specific loss of serotonin from the storage granule. The drastically different effects of suloctidil on different types of membranes could have been a consequence of inherently different characteristics of the membranes. This was indicated by damage to mitochondria while the granule membranes were spared even though both are internal membranes. Damage to plasma membranes could reflect not only inherent characteristics of the membrane but also exposure to a higher initial concentration of the drug.

Mills and Macfarlane [2] have demonstrated a dose-dependent slow liberation of radioactivity from platelets labeled with preabsorbed [^{14}C]serotonin in plasma. A half maximal rate was obtained at a 8 μM suloctidil concentration. The released compounds consisted of serotonin and breakdown products of serotonin, suggesting that suloctidil has a reserpine-like action [2]. Our findings support and extend these observations. By

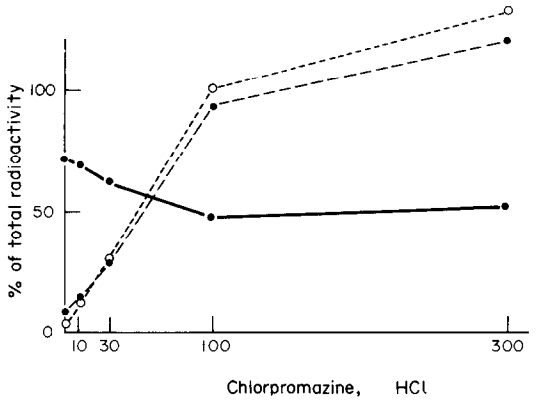

Fig. 11. Lack of effect of incubation with chlorpromazine–HCl on preservation of granular content in the platelet. Experiments were performed with EDTA platelets. Experimental conditions were as described in Materials and Methods and in the legend for Fig. 7. Chlorpromazine–HCl was added in 0.154 M NaCl. Incubation by 5 min shaking at 37°. Key: (●——●), [^{14}C]-ATP in total incubate, (●— — —●) extracellular ^{14}C-labeled nucleotides and (○ · · · ○), extracellular [^{3}H]serotonin as per cent of radioactivity in whole platelets. Mean of two experiments.

excluding soluble proteins from the incubation medium, we obtain a half-maximal rate of serotonin release at 0.6 μM suloctidil and a clear demonstration of effect down to 0.06 μM. This was not accompanied by loss of other stored compounds (LA-PF_4) or adenine nucleotides. Because of this slow leakage of serotonin, the demonstration of retention of granular material in the presence of high concentrations of suloctidil is more clear-cut with LA-PF_4, as can be seen from Fig. 7.

The possibility that suloctidil is a membrane-active drug was further indicated by a comparison of the effects of suloctidil with those of chlorpromazine, an extensively studied membrane-active drug [13, 14]. It has been demonstrated by means of electron microscopy [15] that chlorpromazine incubated with platelet-rich rabbit plasma disrupted platelets and liberated intact granules to the extracellular medium. Similar findings with human platelets are presented by us (Fig. 6). However, our biochemical studies showed that high concentrations of chlorpromazine caused a parallel loss of the cytoplasmic and granule components of the platelets (Fig. 11) while suloctidil caused a selective loss of cytoplasmic constituents. The reason for this may be that the granules liberated by the action of chlorpromazine remain in suspension after centrifugation so that their content cannot be distinguished from material lost from the granules. In contrast, the plasma membrane of suloctidil-treated platelets can be penetrated by cytoplasmic components but not by the granules with their content which will, therefore, sediment with the platelet pellet.

These observations provide new information on the response of platelets to membrane active drugs. However, the relevance of these *in vitro* actions of suloctidil to any *in vivo* action of the drug requires further studies.

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