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# Electrorotation – a new method for investigating membrane events during thrombocyte activation. Influence of drugs and osmotic pressure

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**The measurement of the spin of cells in rotating high-frequency electric fields ('electrorotation') makes possible the investigation of dielectric membrane properties of single cells. This method was applied to membrane permeability changes accompanying thrombocyte activation and compared with light-scattering data. Describing the dielectric behavior of platelets by a single-shell model and assuming a sufficiently low membrane conductivity of $1 \cdot 10^{-7}$ S/m we found for nonactivated platelets a membrane capacity of 5.5 mF/m$^2$ and the conductivity of the internal medium was estimated to be 0.12 S/m. Upon activation, the electrorotation decreased continuously, with half-times in the range of few minutes. This could be explained assuming a 500-fold increase in membrane conductivity. The application of both local anesthetics and virostatics inhibited the decrease of electrorotation, as did hypertonic osmotic pressure. In all cases this was accompanied by inhibition of platelet aggregation. Hypotonic solutions induced self-aggregation and spontaneous loss of electrorotation. It was concluded that the increase in permeability of the granule membrane is a crucial step in the release reaction and that the electrorotation method is able to detect the incorporation of the granule membranes into the plasma membrane during activation. The advantage of this electrorotation method is that it enables measurements on a single-cell level, thus avoiding interactions between platelets.**

## Introduction

The study of membrane events during thrombocyte activation can contribute to an understanding of the mechanisms of the platelet release reaction. The basic biophysical mechanisms underlying the activation process are not very well understood. This is due, at least in part, to experimental difficulties having to do with such factors as very fast reactions, instability of platelet suspensions, etc.

One of the main biophysical problems concerns the mechanism by which different stimuli induce the exocytotic release reaction. One idea is that at later stages biochemical signals are transformed into permeability changes [1]. There is considerable evidence that the activation process of thrombocytes and membrane permeability changes are strongly connected [2–4]. Pollard and coworkers [2,5,6] have suggested that gradients represent essential driving forces in completing membrane exocytotic reactions. They conclude that the anion permeability of granule membranes increases, thus leading to ion equilibration and extra colloid-osmotic pressure differences. This hypothesis is supported by the findings that hyper-

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tonic osmotic pressure and anion permeability blockers inhibit platelet activation.

Through his studies of the fusion of lipid vesicles with planar lipid membranes, Zimmerberg [7] was able to demonstrate, even in an artificial system, a possible role of osmotic gradients as driving forces for fusion. Similarly, chromaffin granule membrane fusion was shown to be inhibited by hypertonic solutions [2,5]. At present, there is still disagreement about the ionic species involved in the permeability increase which leads to granular swelling. Pollard proposed an increase of hydroxyl ion permeability [2], whereas Grinstein [8] emphasized the role of protons, eventually similar to a proposed proton-cation exchange mechanism for chromaffin granule swelling [9].

One of the possible experimental approaches to studying those permeability changes possibly involved in the release reaction is the use of fluorescent indicator dyes to detect associated electric potential changes [3,8]. This method, however, requires relatively high platelet concentrations and is in essence indirect. An advantage is that it becomes possible to identify the ionic species involved through the use of specific carriers or channels.

An alternative is the use of the new method of electrorotation [10–13] to investigate membrane permeability changes during exocytosis more directly [14,15]. Of course, the main disadvantage of this method is that the membrane electrical conductivity is measured, thus making it difficult to single out the particular ion species responsible for a conductivity change.

Electrorotation is the result of a polarization phenomenon which is induced by charge separation and dipole orientation at the interfaces of dielectrics. The inertia of the polarization creates with increasing field frequency an angle between the electric field vector and polarization. The resulting dipole moment has components directed either in the electric field vector, or opposite to it, thereby inducing repulsive or attractive forces. These cause the object to rotate either against or with the rotating field.

With other systems this technique has recently been shown to be a valuable method for studying conductivity changes of single cells [12,13]. Electric membrane properties of erythrocytes, plant cells and protoplasts have been measured successfully [13–15]. With this new method, 'electrorotation', we wished to study in particular the influence of osmotic pressure changes and of various drugs by comparing electrorotation data with the results of the classical light-scattering method for detecting platelet aggregation.

## Materials and Methods

*Preparation of platelets*

Platelets for aggregation studies and electrorotation measurements were prepared from freshly drawn venous blood. The blood was collected through siliconized needles leading into siliconized glass tubes and was then anticoagulated with sodium citrate in a proportion of 1 part 3.8% (w/v) unbuffered sodium citrate per 9 parts blood. Platelet-rich plasma with a count of $((3.5 \pm 0.4) \cdot 10^5)$ platelets per $\mu$l was prepared by centrifugation of the blood samples at $200 \times g$ for 10 min at 4°C after 1 h incubation at room temperature. Platelet-poor plasma was obtained by means of centrifugation with $300 \times g$ for 10 min at 4°C. It contained less than $10^3$ platelets per $\mu$l.

*Platelet aggregation*

Platelet aggregation was studied by means of an aggregometer (KZM-1, Labortechnik Ilmenau, G.D.R.) according to the method of Born [16].

The extent of aggregation was determined by measuring the percentage difference in light transmission between platelet-rich and platelet-poor plasma after 5 min stirring at 1000 rpm and 37°C.

The measuring tubes were filled with 500 $\mu$l platelet-rich plasma. After 2 min stirring, 20 $\mu$l containing the aggregation-inducing substance was added. The sensitivity of the samples to ADP and collagen was determined in preliminary experiments. We used collagen (Collagenreagent Horm, Hormon-Chemie, München, or Test-Collagen, Behring-Werke AG, Marburg, F.R.G.) in concentrations between 0.25 and 4.0 $\mu$g/ml and ATP (Reanal. Budapest, Hungary) in concentrations between 0.31 and 5.0 $\mu$M, respectively.

The influence of the following drugs was investigated: sodium or potassium salts of 1-adamantanecarboxylic acids (Institute of Electro-

chemistry of the Academy of Sciences of the U.S.S.R., Moscow), rimantadine ($\alpha$-methyl-1-adamantanemethylamine hydrochloride) (Institute of Organic Chemistry of the Academy of Sciences, LSSR, Riga), chlorpromazine (Sigma), indomethacin (Sigma) and tetracaine (Sigma). Not more than 20 $\mu$l of stock drug solution was added to 500 $\mu$l of platelet-rich plasma and then incubated at 37°C for 5 min without stirring. The threshold concentration of ADP was then added and the aggregation of platelets was determined as described above.

*Electrorotation*

We used a rectangular measuring chamber with four V2A-steel electrodes with the dimensions 6.3 $\times$ 4.0 $\times$ 1.0 mm, chamber diameter 2.2 mm. Instead of coverslip, a very thin transparent foil (Frapan) was used. The volume of the chamber was approx. 5 $\mu$l. A thrombocyte concentration of 200 platelets/$\mu$l was used. This prevented to the greatest possible extent interaction between platelets. A 10 mM Tris-buffered 300 mM sucrose solution (pH 7.4) was used. The resulting measuring solution had conductivities of 10–22 mS/m. The electrorotation measurements were performed at 22°C. It generally took 8–10 min to complete a full measuring cycle with a single thrombocyte. In the electrorotation experiments we used thrombin (Arzneimittelwerk Dresden, G.D.R.) in a concentration of 0.4 U/ml ionophore and A23187 (Calbiochem, Behring Corp., 0.3 mM) as activators. Each individual platelet was measured at the following frequencies (MHz): 0.056, 0.1, 0.18, 0.56, 1.0, 6.5 and 8.0. The last two frequencies were used to determine the intersection of the rotation with the abscissa, since between these two frequencies the direction of rotation was reversed. A more detailed description of theory and data evaluation is given in Appendix.

## Results

Fig. 1 demonstrates that thrombocytes do indeed generally show the theoretically predicted electrorotation behavior as a function of the frequency of the rotating electric field applied (Eqn. A1). This result was used to estimate the electrical parameters of untreated platelets.

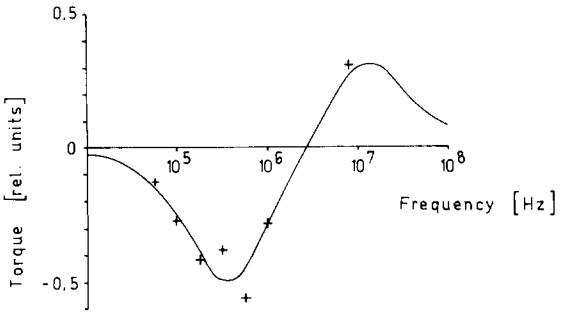


Fig. 1. Dimensionless rotation (measured angular velocity of platelets divided by the square of the field strength) of a single untreated thrombocyte as a function of frequency (representative sample). 300 mosM sucrose/10 mM Tris (pH 7.4). The solid line is a theoretical curve according to Eqns. A1–A2g with the following parameters: $r = 0.89\ \mu$m, $\varepsilon_m = 7.07$, $\varepsilon_e = 80$, $\varepsilon_i = 50$, $G_e = 0.012$ S/m, $G_m = 10^{-7}$ S/m, $G_i = 0.105$ S/m, $d = 8$ nm.

For each individual platelet we determined the first maximum frequency, performing a two-parameter fit according to Eqn. A5 and the frequency corresponding to zero rotation by using Eqn. A3. Then, assuming the model of a sphere surrounded by a thin membrane shell, we determined directly the membrane dielectric constant and the conductivity of the interior for each individual platelet by solving the system two equations, A2g and A4. Since there are three unknowns (membrane dielectric constant, internal conductivity and membrane conductivity) and only two equations, we had to assume certain values for one of these parameters.

An analysis of the influence of the membrane conductivity on the two other parameters showed that the membrane dielectric constant and the internal conductivity were practically unaffected by membrane conductivity as soon as the latter was small (Fig. 2). The range of membrane conductivity at which it was practically without influence on the calculated membrane dielectric constant and the conductivity of the interior was just characteristic for plasma membranes. This enabled us to estimate values for the two other parameters by processing the data of 30 thrombocytes within the limitations of the single-shell model used. The procedure indicated above yielded the parameters given in Table I.

Now we were interested in how the electrorota-

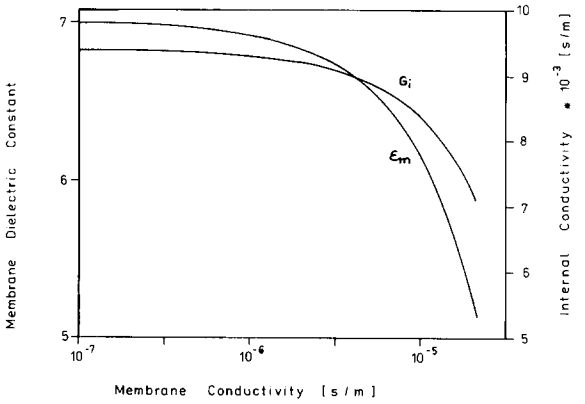


Fig. 2. Internal conductivity and membrane dielectric constant as a function of membrane conductivity according to Eqns. A2g and A4. The curves correspond to the data of a representative thrombocyte. The experiment yielded $f_{c1} = 0.331$ MHz and $f_0$-2.41 MHz obtained with Eqns. A5 and A3. We assumed further $d_m = 8$ nm, $\varepsilon_e = 80$, $\epsilon_i = 50$. The radius was 0.44 $\mu$m and the external conductivity was $5.4 \cdot 10^{-3}$ S/m.

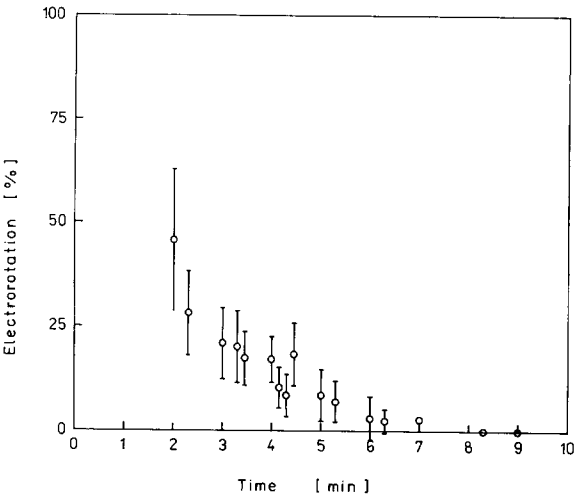


Fig. 3. Rotation of thrombin-activated platelets (0.4 U/ml) as a function of time. 300 mosM sucrose/10 mM Tris (pH 7.4). The conductivity ranged from $5.4 \cdot 10^{-1}$ to $6.2 \cdot 10^{-3}$ S/m. Frequency of applied field was 0.56 MHz. S.E. given.

tion behavior changed after activation. To obtain a mean value for the first characteristic frequency, additional untreated platelets, all from the same donor, were measured. For about 40 platelets the mean value of the first characteristic frequency ($f_{c1}$) was $0.523 \pm 0.14$ MHz. The oscillator was adjusted to a fixed frequency, $f = 0.56$ MHz, close to $f_{c1}$, and the thrombocyte rotation during activation was followed as a function of time. Fig. 3 shows that the thrombin-induced activation was accompanied by a continuous decrease in electrorotation with a characteristic half-time value of about 2 min. After about 8 min the rotation of thrombocytes ceased completely. It was also confirmed that over the whole range of frequencies investigated (0.056–1.0 MHz) no rotation was observable.

With A23187 activation, the same behavior of electrorotation was found, but the decrease in electrorotation was about 3-times faster than with thrombin-induced activation. In this context, the aggregation reaction after activation was measured. Fig. 3 shows the aggregation as a function of time after addition of ADP. If one compares the half-time of the decrease in thrombin-induced electrorotation and the increase in aggregation, it is seen that both time constants are very close.

After convincing ourselves that the decrease in electrorotation was closely related to aggregation after activation, we were interested in whether

TABLE I

PARAMETERS OF UNTREATED THROMBOCYTES

Medium: 300 mosM sucrose/10 mM Tris (pH 7.4); $n = 30$, $\pm$S.E.

| MEasured values | Assumed parameters | Calculated parameters |
|---|---|---|
| External conductivity: $G_e = 12 \cdot 10^{-3}$ S/m | membrane conductivity: $G_m = 10^{-7}$ S/m | internal conductivity: $G_i = 0.117 \pm 0.028$ S/m |
| First peak frequency: $f_{c1} = 0.488 \pm 0.129$ MHz | membrane thickness: $d = 8.0$ nm | membrane dielectric constant: $\varepsilon_m = 4.8 \pm 1.2$ |
| Frequency of zero rotation: $f_0 = 2.5 \pm 0.454$ MHz | external dielectric constant: $\varepsilon_e = 80$ | specific membrane capacity: $C_m = (5.5 \pm 1.2) \cdot 10^{-3}$ F·m$^{-2}$ |
| Radius of platelets: $r = 0.89 \pm 0.2$ $\mu$m | | |

TABLE II

QUALITATIVE MEASUREMENT OF THE INHIBITION OF THE ROTATION DECREASE BY DRUGS

Field frequency was 0.56 MHz. 300 mosM sucrose/10 mM Tris (pH 7.4), ±S.E. In the control (nonactivated platelets) nearly all thrombocytes rotated.

| Drug concentration | Rotating thrombocytes (%) | Activator |
|---|---|---|
| None | 0 | thrombin |
| Amantadine, 3.0 mM | 82.4±4 ($n$=18) | (0.4 U/ml) |
| 1-Adamantanecarboxylic acid, 3.0 mM | 80.6±8 ($n$=12) | |
| Rimantadine, 3.0 mM | 78.8±6 ($n$=30) | |
| Chlorpromazine, 0.3 mM | 82.1±7 ($n$=15) | |
| None | 0 | A23187 |
| Amantadine, 3.0 mM | 74.9±8 ($n$=10) | (0.3 mM) |
| 1-Adamantanecarboxylic acid, 3.0 mM | 81.8±7 ($n$=10) | |
| Rimantadine, 3.0 mM | 85.0±6 ($n$=10) | |
| Chlorpromazine, 0.3 mM | 92.2±4 ($n$=10) | |

known inhibitors of aggregation would also inhibit the decrease in electrorotation. Table II shows that with a number of drugs which are known to inhibit aggregation [17–19], the decrease in electrorotation was also inhibited. We were interested in determining whether the $Ca^{2+}$-ionophore A23187-induced rotating response, i.e., the decrease of thrombocyte rotation after application of the ionophore can be also inhibited. Table II shows the result. The same drugs which inhibit the decrease in the rotation after addition of thrombin also inhibited the ionophore-induced rotation response. There was no difference in the effects of the applied drugs with respect to both activators used. Since it was found that aggregation was partly reversible when these drugs were added after activation [20], we tested whether this would be also the case for the electrorotation decrease. Thrombocytes were activated with A23187 (0.3 mM): after 5 min the drugs (3.0 mM amantadine, 3.0 mM 1-adamantanecarboxylic acid, 3.0 mM rimantadine) were added. A single experiment demonstrates that 30% of the nonrotating thrombocytes (after the A23187 activation reaction) again started to rotate but apparently with lower speed. Since Pollard [2] has shown that high extracellular osmotic pressure inhibits the serotonin release reaction from plate-

TABLE III

QUALITATIVE MEASUREMENT OF THE ROTATION BEHAVIOR OF THROMBOCYTES AS A FUNCTION OF HYPOTONIC OSMOTIC PRESSURE

Rotation measurements at 0.56 and 1.0 MHz, 10 mM Tris (pH 7.4). Various sucrose concentrations to adjust the osmotic pressure.

| Osmotic pressure (mosM) | Percentage of rotation platelets (%) | Rotation after thrombin (%) | Remarks |
|---|---|---|---|
| 250 | 100 | 0 | forming |
| 200 | 100 | 0 | pseudopods |
| 175 | 60 | 0 | slow |
| 150 | 30 | 0 | rotation |

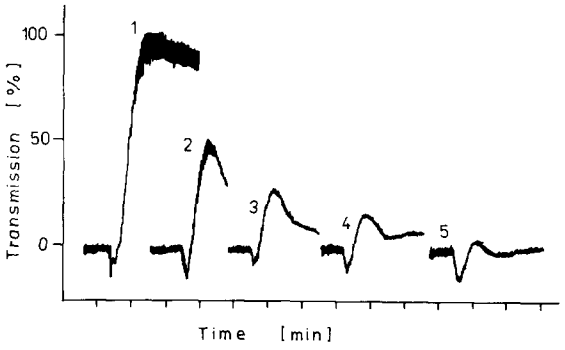


Fig. 4. Aggregation of thrombocytes as a function of time under presence of indomethacin. ADP concentration was 1.25 mM. Curve 1, without drug (aggregation reaction corresponded to 100%); curve 2, 0.3; curve 3, 0.4; curve 4, 0.5; curve 5, 0.7 mM indomethacin. More details in Materials and Methods.

lets. we were interested to see whether the electrorotation behavior also remained unchainged. Under hypertonic conditions of 1000 mosM (sorbit solution) no change of thrombocyte rotation was observed after thrombin activation (0.4 U/ml).

To our knowledge the influence of hypotonic extracellular media has not been investigated systematically. Ulutin [21] reported a possible spontaneous activation of thrombocytes in platelet-rich plasma by means of dilution which distilled water. He did not, however, give detailed information about the osmotic conditions. Our rotation experiments (Table III) showed that low osmotic pressure decreased the percentage of rotating thrombocytes. While 100% of thrombocytes still rotated at 250 mosM and 200 mosM, below 175 mosM the number of rotating cells was drastically reduced. Yet, the remaining rotating platelets could be still stopped by adding thrombin. Even in the range of self-aggregation (150 mosM) we still found rotating platelets, but at a lower speed, which also could be finally stopped by adding thrombin. These observations indicate that the activation system seems still to be still.

By means of the light-scattering technique, a graduated increase as in the activation was observed as a function of the osmotic pressure of the extracellular medium. This is illustrated in Table IV. No activation was seen in 284 mosM at the weak activator concentrations employed. We explained this by the dilution of the active plasma constituents to achieve hypotonic osmotic pressures of the suspending medium. But at still lower osmotic pressure less and less activator was necessary to induce aggregation.

TABLE IV

AGGREGATION AS A FUNCTION OF REDUCED OSMOTIC PRESSURE

Transmission was used to quantify aggregation. Approx. 14 cm corresponded to complete aggregation under physiological conditions. 100 $\mu$l of $10^6$ platelets/$\mu$l containing concentrate were added to 400 $\mu$l of different NaCl concentrations.

| Osmotic pressure (mosM) | Transmission (cm) | Activator |
|---|---|---|
| 284 | 0 | 5 $\mu$M ADP, 2.5 $\mu$M $Ca^{2+}$ |
| 217 | 11.7 | 5 $\mu$M ADP, 2.5 $\mu$M $Ca^{2+}$ |
| 181 | 12.8 | 5 $\mu$M ADP, 2.5 $\mu$M $Ca^{2+}$ |
| 143 | 12.0 | 2.5 $\mu$M $Ca^{2+}$ |
| 123 | 13.5 | none |

## Discussion

The analysis of the electrorotational behavior (Eqn. A1) revealed that the existence of the first characteristic frequency, $f_{c1}$, was strongly related to the dielectric properties of the membrane, whereas the second characteristic frequency $f_{c2}$ was mainly determined by the electric properties of the internal and external media, since at very high frequencies the membrane is short-circuited by its capacity [13]. At the two characteristic frequencies the torque has extreme values. If the membrane conductivity increases significantly the rotation decreases. Simultaneously, the maximum frequency, $f_{c1}$, is slightly shifted towards higher values. This behavior is illustrated in Fig. 5. The curves are shown as a function of the membrane conductivity, all other parameters being kept constant. Behavior as in Fig. 5 cannot be simulated varying other parameters (cf. Ref. 13). From Fig. 5 it follows that the torque and the first characteristic frequency are only slightly influenced when the membrane conductivity is increased from 0.1 to 10 $\mu$S/m. A drastic decrease of the torque is observed when the membrane conductivity ex-

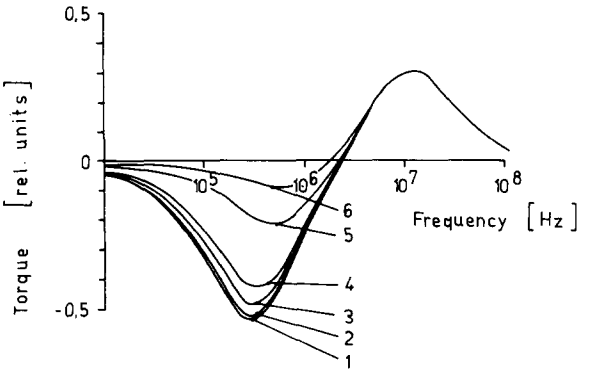


Fig. 5. Theoretical simulation of the frequency dependence of rotation of thrombocytes for various assumed membrane conductivities. Curves 1–6 correspond to the following membrane conductivities (S/m): $1 \cdot 10^{-7}$; $1 \cdot 10^{-6}$, $5 \cdot 10^{-6}$, $1 \cdot 10^{-5}$, $5 \cdot 10^{-5}$, $1 \cdot 10^{-4}$. According to Eqns. A1–A2g, the following parameters remained constant: $r = 2.2$ $\mu$m, $d = 8$ nm, $\varepsilon_e = 80$, $\varepsilon_m = 2.89$, $\varepsilon_i = 50$, $G_e = 9.3\ 10^{-3}$ S/m, $G_i = 0.096$ S/m.

ceeds 100 μS/m, while the first characteristic frequency is shifted by only 19% to higher frequency [13]. If the conductivity of the extracellular medium is smaller, the range of detectability of membrane conductivity changes towards lower conductivities can be increased. As a consequence, membrane conductivities above 50 μS/m could not be estimated precisely, because the first maximum became too small to be detectable within experimental error under our conditions. The main point is that in the present study the method of thrombocyte preparation did not allow any further reduction in the conductivity of the bulk solution. Nevertheless, all in all, we could draw the conclusion that activated thrombocytes should be characterized by membrane conductivities at least 500-times higher than untreated platelets, because we observed complete inhibition of rotation. We are aware of the limitations and shortcomings involved in determining dielectric parameters of platelets by using electrorotation data.

Neither did the theory of electrorotation applied take into account the ellipsoidal shape, the membrane surface changes by pseudopods upon activation, and the presence of surface charges. Other simplifications and assumptions, e.g., not knowing the precise thickness of the membrane, are presented in Table I. Nevertheless, since the experimentally observed behavior of the platelets nicely corresponded to the theoretically predicted behavior (a single peak at low frequencies), we think that our estimated data are worth presenting, together with the assumptions. Fluorescence studies by Boudet et al. [1]. Feinmann and Detwiler [4], Greenberg-Spersky and Simons [3], and Varecka and Pogady [22] as well as studies on the release reaction of different substances [3,19] have suggested an increase in the membrane permeability during thrombocyte activation. This permeability increase was indirectly confirmed here, since the conductivity increased dramatically.

This increase could bear a relationship to the suggested osmotic mechanism of swelling of the granule during exocytosis. The results of increase in permeability we have presented and, consequently, of ion equilibration [2,6] support the osmotic hypothesis. We are inclined to believe that we were measuring the permeability change of the outer plasma membrane induced by incorporation of the more permeable granule membranes. Pollard [2] was able to measure an inhibition of exocytosis by applying increased osmotic pressure. Our electrorotation measurements confirmed this result on the level of membrane conductivity. Exocytosis is perhaps always accompanied by subsequently high plasma membrane permeability. It is probably the case that, with increasing osmotic pressure of the cytoplasma, the granule cannot take up enough water to swell and its membrane is not incorporated into the plasma membrane. In contrast, decreased osmotic pressure should result in granule swelling without permeability increase and one would eventually expect spontaneous activation. Indeed, Ulutin [21] found self-activation when diluting the platelet suspensions with water. Since this result could also be explained by lysis of platelets, we tried to demonstrate this effect by a more graduated decrease of the osmotic pressure. The electrorotation experimental arrangement had the advantage of very low platelet concentrations. Therefore, our finding of a gradual decrease of electrorotation accompanied by a gradual increase in aggregation makes the idea of activation induced by release of activators following lysis of some thrombocytes less attractive.

The inhibition of aggregation induced by local anesthetics and virostatics was always correlated with a loss of the time response of rotation after activation. Consequently, these drugs inhibited the membrane permeability increase. By analogy to the effect of anesthetics on nerve excitation, we think that the drugs prevented the permeability increase of the granula membrane responsible for swelling, due to an unspecific influence on channels. Consequently, neither the release reaction nor the aggregation would take place.

The drugs inhibited not only the activation response induced by physiological activators like thrombin, but also that induced by the calcium ionophore A23187. This was measured by both light scattering and electrorotation and is in agreement with fluorescence studies (Boudet et al. [1]; Feinstein et al. [19]). Another finding supporting the idea of drugs affecting ionic channels is that we indeed could demonstrate the partly reversible character of drug action on the electrorotation behavior. In the present paper we have

shown that electrorotation experiments can provide valuable information on membrane processes during thrombocyte activation. Most importantly, investigations on the single-cell level became possible. Currently, the use of this technique for studies with pathological thrombocytes in medical practice is being tested.

**Acknowledgements**

We thank Dr. G. Fuhr and J. Gimsa for valuable suggestions concerning the electrorotation technique. The medical staff of the blood-clotting laboratory of the Charite generously supported this work, even though it interfered with their routine requirements.

## Appendix

A comprehensive theoretical approach was first presented by Fuhr and co-workers [13–15]. Sauer and Schlögel [23] used a different approach to describe electrorotation phenomenon. In the measurable rotation range of biological objects, both theoretical approaches give the same result. We applied the model of a sphere surrounded by a thin shell (membrane) to thrombocyte electrorotation data. This model was discussed by Glaser and Fuhr [13,21]. Rotation experiments performed at different electric field strengths become comparable if the rotation, $R$, is introduced means of dividing the measured angular velocity by the square of the electric field strength, $E$. The order of magnitude of $R$ observed was $10^{-8}$–$10^{-7}$ rad · $m^2 \cdot V^{-1} \cdot s^{-2}$. According to the assumed model the torque, $N$, reads [13,14]:

$$N = 4\pi\varepsilon_0\varepsilon_e r_2^3 E^2 \left[ \left( \frac{C_1}{C_2} - \frac{B_1}{B_2} \right) \frac{\frac{f}{f_{c1}}}{1+\left(\frac{f}{f_{c1}}\right)^2} + \left( \frac{B_1}{B_2} - \frac{A_1}{A_2} \right) \frac{\frac{f}{f_{c2}}}{1+\left(\frac{f}{f_{c2}}\right)^2} \right] \quad \text{(A1)}$$

$f_{c1}$ and $f_{c2}$ are the frequencies, $f$, of the applied field corresponding to the maximum rotation (characteristic frequencies). $r_1$, $r_2$ are the inner and outer radia of the membrane-coated particle, $\varepsilon_0$ is the absolute permittivity of vacuum and $\varepsilon_e$ is the relative dielectric constant of the external medium. The parameters $A_1$, $A_2$, $B_1$, $B_2$, $C_1$, $C_2$ are defined as follows:

$$A_1 = \varepsilon_m(\varepsilon_e - \varepsilon_i) + \frac{d}{r_2}(\varepsilon_i - \varepsilon_m)(2\varepsilon_m + \varepsilon_e); \text{ where } d = r_2 - r_1 \quad \text{(A2a)}$$

$$A_2 = -\varepsilon_m(\varepsilon_i + 2\varepsilon_e) - \frac{2d}{r_2}(\varepsilon_i - \varepsilon_m)(\varepsilon_e - \varepsilon_m) \quad \text{(A2b)}$$

$$B_1 = -G_m(\varepsilon_e - \varepsilon_i) - \varepsilon_m(G_e - G_i) - \frac{d}{r_2}[(G_i - G_m)(2\varepsilon_m + \varepsilon_e) + (2G_m + G_e)(\varepsilon_i - \varepsilon_m)] \quad \text{(A2c)}$$

$$B_2 = G_m(\varepsilon_i + 2\varepsilon_e) + \varepsilon_m(G_i + 2G_e) + \frac{2d}{r_2}[(\varepsilon_i - \varepsilon_m)(G_e - G_m) + (\varepsilon_e - \varepsilon_m)(G_i - G_m)] \quad \text{(A2d)}$$

$$C_1 = -G_m(G_e - G_i) - \frac{d}{r_2}(G_i - G_m)(2G_m + G_e) \quad \text{(A2e)}$$

$$C_2 = G_m(G_i + 2G_e) + \frac{2d}{r_2}(G_i - G_m)(G_e - G_m) \quad \text{(A2f)}$$

Here $G$ denotes the conductivity and $\varepsilon$ the dielectric constant. The indices e, i, m stand for external medium, internal medium and membrane respectively. The first characteristic frequence is given by:

$$f_{c1} = \frac{C_2}{\varepsilon_0 B_2} \quad \text{(A2g)}$$

The measured parameter rotation is for stationary slow rotations directly proportional to the torque $N$ (Eqn. A1) divided by the square of the electric field strength. The frequency of the applied field resulting in zero rotation of particles, $f_0$, is an important parameter and was obtained by measuring the platelet rotation at two frequencies, yielding opposite rotation directions, namely at 1.0 MHz and at 6.5 or 8.0 MHz. Then $f_0$ was de-

termined by the linear approximation:

$$\lg f_0 = (R_1 \lg f_2 - R_2 \lg f_1)/(R_1 - R_2) \quad \text{(A3)}$$

$R_1$ and $R_2$ denote the rotation at the applied field frequencies $f_1$ (1.0 MHz) and $f_2$ (6.5 MHz or 8.0 MHz). On the other hand, $f_0$ is given explicitly by:

$$f_0 = \left\{ \frac{C_2^2(A_2B_1 - A_1B_2) + B_2^2(B_1C_2 - B_2C_1)}{4\pi^2\varepsilon_0^2\left[A_2^2(B_2C_1 - B_1C_2) + B_2^2(A_1B_2 - A_2B_1)\right]} \right\}^{1/2} \quad \text{(A4)}$$

The first characteristic frequency, $f_{c1}$, is given by Eqn. A2g with Eqns. A2d and A2f. At sufficiently small frequencies, $f \ll f_{c2}$, the theoretical curve reduces to a single bell-shaped curve:

$$R = \frac{2R_{max}\frac{f}{f_{c1}}}{1 + \left(\frac{f}{f_{c1}}\right)^2} \quad \text{(A5)}$$

where $R_{max}$ is the rotation $R$ at the first characteristic frequency, $f_{c1}$. A least-square fit according to Eqn. A5 was used to determine $f_{c1}$ from the experiment.

From the experimentally derived parameters, $f_0$ and $f_{c1}$, it will be then possible to calculate the membrane dielectric constant and the conductivity of the internal medium, when assuming certain realistic values for internal and external permittivity and inserting a sufficiently small membrane conductivity [13]. The membrane capacity, $C_m$, was finally evaluated using the membrane thickness, $d$, and the membrane dielectric constant assuming a flat plate condenser model.

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