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Permeability Changes Induced by Electric Impulses in Vesicular Membranes

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Summary. Electric impulses were found to cause transient permeability changes in the membranes of vesicles storing biogenic amines. Release of catecholamines induced by electric fields (of the order of 20 kV/cm and decaying exponentially with a decay time of about 150 μsec) was studied, using the chromaffin granules of bovine adrenomedullary cells as a vesicular model system. Far-UV-absorption spectroscopy was applied to determine the amount of catecholamines released from suspended vesicles. A polarization mechanism is suggested for the induction of short-lived permeability changes caused by electric fields. Such transient changes in permeability may possibly represent a part of the sequence of events leading to stimulated neurohumoral secretion.

It is a well-known neurophysiological observation that neurohumoral secretion rises strongly during nerve stimulation. Nerve activity in general, and nerve impulses in particular, accelerate the release of hormones and transmitter substances from their storage compartments (Bloom, Iversen & Schmitt, 1970; review).

Electron-microscopic studies have shown that vesicular structures accumulate near secretory cell junctions and nerve terminals. These vesicles are, in general, spherical particles bounded by a membrane, and contain biogenic amines in very high concentration. It has therefore been suggested that nerve activity causes increased release of neuroamines from these storage vesicles. However, the mechanism of these electro-secretory processes is not known (von Euler, 1970). One possible step of this mechanism may consist in a direct action of nerve impulses on vesicles adjacent to nerve membranes, for instance in the synaptic terminal. The release of biogenic amines from a vesicular system *in vitro* by electric impulses, was therefore investigated.

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In the present study, the chromaffin granules of bovine adreno-medullary cells are used as a vesicular model system. The main soluble constituents of these vesicles are epinephrine (E), norepinephrine (NE), ATP and proteins. Upon electric stimulation of the gland, these substances are released into the extracellular space in the same molar ratio as that found in the intact granules (Smith, 1968; review). A spectroscopic method, based on absorption flattening (Duysens, 1956) in the far-UV range is introduced, permitting the quantitative determination of catecholamine release from suspended granules.

The result of electric impulse experiments with vesicle suspensions are reported and a mechanism for the transient permeability changes in the vesicle membranes is suggested.

Materials and Methods

Preparation of Vesicle Suspensions

Chromaffin granules were prepared from bovine adrenal medullae by the method of isotonic density gradient centrifugation (Trifaro & Dworkind, 1970), which yields relatively pure, intact granules, suitable for *in vitro* release studies. The procedure, carried out at about 4 °C, consists of homogenization of the medullae in 0.27 M sucrose, removal of unbroken cells, debris and nuclei by centrifugation at $1{,}200 \times g$ for 10 min, and pelleting of the granules by a second centrifugation at $24{,}000 \times g$ for 10 min. The granule pellet, still containing mitochondria and lysosomes, is resuspended in 0.27 M sucrose, layered on top of a solution of 0.27 M sucrose and 19.5% (w/v) Ficoll in D_2O, and centrifuged at $100{,}000 \times g$ for 60 min. The pellet containing very pure granules is washed with 0.27 M sucrose, resuspended in 0.27 M sucrose-0.01 N NaCl, pH ~6.5, and stored at 0 °C. The volume fraction F of the vesicles in suspension can be obtained from the relation $F = m/(\rho_v p)$, where m is the protein concentration in g/cm^3, ρ_v the density of the vesicle and p the weight fraction of protein. Here, $m = 0.05\ (\pm 0.01)$ mg/cm^3, as determined by the method of Lowry, Rosenbrough, Farr and Randall (1951), and $p = 0.115$ (Smith, 1968). The vesicle density is obtained from density gradient centrifugation; $\rho_v = 1.202$ g/cm^3 (which is the density of sucrose-Ficoll-D_2O). Thus, $F = 3.6\ (\pm 0.72) \times 10^{-4}$.

Spectroscopic Method

The spectra in the far-UV range are measured with a Cary 15 spectrophotometer, using quartz cells of 0.1 cm light path. The accuracy of the optical density recording is 0.005.

The absorption spectrum of a vesicle suspension between 195 and 270 nm is shown in Fig. 1. The shape of this spectrum is determined by the contributions of the main constitutents of the vesicles: catecholamines (CA), ATP, soluble proteins, membrane (insoluble) proteins and lipids. From the known vesicle composition (Smith, 1968), the contribution of CA and ATP is calculated to be about 75% of the absorbance at the maximum, i.e., 198 (± 0.5) nm. The spectrum is, however, distorted with respect to the spectrum of a molecularly dispersed solution of the same components (at the same con-

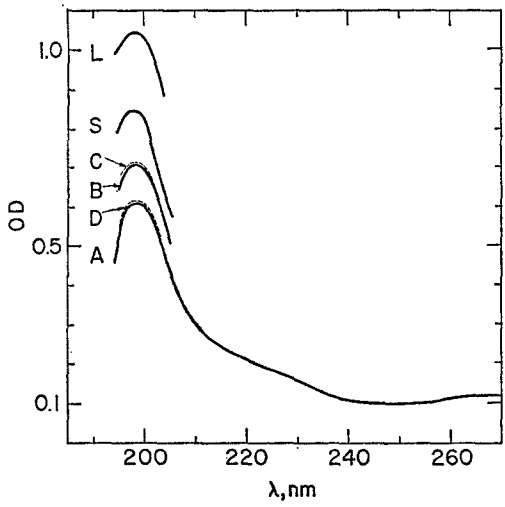

Fig. 1

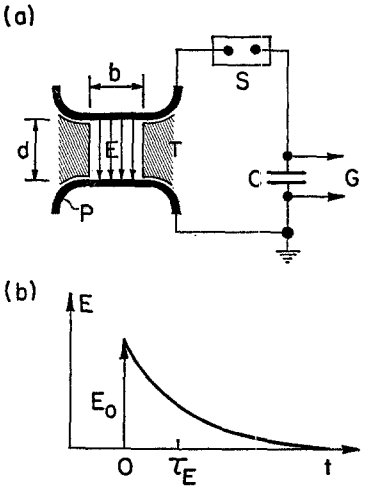

Fig. 2

Fig. 1. Far-UV absorption spectra of vesicle suspensions (optical density, OD per mm path length). *A* at 0 °C; *D* at 23 °C, 1 min after exposure to this temperature; *B* at 0 °C, after an impulse (~20 kV/cm); *C* as *B*, 1 min after exposure to 23 °C; *S* saturation of thermal release at 23 °C; *L* after osmotic lysis of vesicles in H_2O

Fig. 2. (*a*) Scheme of the impulse device. *T* Teflon block; *P* platinum electrode; *d* height, and *b* length of the square cavity filled with solution to be studied; *E* direction of the homogeneous electric field; *S* spark gap (trigger); *C* high-voltage storage capacitor; *G* high-voltage generator. (*b*) Electric impulse. E_0 initial field intensity; *t* time; τ_E relaxation time of the field decay; *see* Eq. (1)

centrations). This is caused by the scattering effects, arising from the particulate nature of the system: the optical densities (OD) at longer wavelengths are increased and the OD of the maximum in the far-UV range is decreased.

After release takes place, the spectrum of the suspension changes. In particular, the OD at 198 nm increases appreciably since the vesicular constituents released into the solution are no longer subject to the scattering artifacts. We may therefore follow the extent of CA release from suspended vesicles by measuring the change in OD; e.g. at the maximum at 198 nm. For this purpose, the spectra of the suspensions are recorded between 195 and 205 nm, using a scanning velocity of 1 nm/sec.

Impulse Method

The solution to be studied is placed between the two flat, platinum-covered electrodes of a thermostated Teflon cell. The cell is part of a high-voltage discharge circuit, schematically represented in Fig. 2. (*See also* Eigen and De Maeyer, 1963.) At time zero, the high voltage storage capacitor *C* is discharged *via* a spark gap and thereby the voltage V_0 is applied to the electrodes of the cell. The finite resistance *R* of the solution causes a decay of the initial field intensity $E_0 = V_0/d$, where $d = 0.84$ cm is the distance between the electrodes.

Because of the very low inductance of the discharge circuit, the dependence of the field decay on time t is given by

$$E(t)=E_0\exp(-t/\tau_E) \tag{1}$$

where the relaxation time of the field decay is $\tau_E=RC$. At 0 °C, the temperature of the experiments, the resistance of the filled cell is $R=3{,}030\ \Omega$. Since $C=5\times10^{-8}$ F, $\tau_E=151.5$ μsec. The field decay of an impulse is shown in Fig. 2.

After the voltage V_0 is applied, the electric field is established instantaneously. The dissipation of the electric energy during the decay of the field occurs within picoseconds; thus, no local overheating can occur.

The total change in temperature caused by Joule heating is given by

$$\Delta T=CV_0^2/(8.36c_p\rho b^2 d)$$

where c_p is the specific heat of the suspension – $c_p\simeq1$ cal/g deg – and b^2d is the volume $v\simeq0.4$ cm^3. The density of the vesicle suspension is $\rho=1.05$ g/cm^3. If V_0 is measured in kV/cm, we obtain $\Delta T\simeq1.43\times10^{-2}\,V_0^2$. The largest voltage applied in our experiments is 20 kV; thus the corresponding temperature increase amounts to about 6 °C.

The rise-time of the temperature increase is given by $\tau_T=RC/2$. The rapid heating leads to an initially isochoric pressure change propagating as a shock wave. In our case, the propagation time of the pressure wave per electrode distance is about 12 μsec. Since this is smaller than $\tau_T=76$ μsec, no cavitation effects (Eigen & De Maeyer, 1963) can occur. Thus, this potential source for vesicle destruction can be eliminated.[1]

Results

Determination of Thermal Efflux

Changes in the optical density ΔOD caused by release of vesicular substances, are correlated with changes in the concentration of CA and ATP liberated into the suspension. We may therefore calibrate, for instance, $\Delta\mathrm{OD}_{198}$ in terms of absorbance changes ΔA_{198} caused by variations in the concentration of "free" CA and ATP. The calibration is performed as follows: aliquots of the stock suspension of vesicles (stored at 0 °C) are incubated at 23 °C (at time zero). After various time intervals $\Delta t_i=t_i-t_0$, the values of $\mathrm{OD}_{i,198}$ at 23 °C are measured. Then each vesicle sample is quickly brought to 0 °C and centrifuged at $48{,}000\times g$ for 10 min, and the absorbance $A_{i,198}$ of the (vesicle free) supernatant is measured. We calculate $\Delta\mathrm{OD}_{i,198}$ as the difference between $\mathrm{OD}_{i,198}$ measured after Δt_i and OD_{198} of the original suspension at 0 °C, corresponding to zero concentration of "free" CA and ATP. The results of such a series of measurements are plotted in Fig. 3. A linear relationship is obtained:

$$\Delta A_{198}=f\Delta\,\mathrm{OD}_{198} \tag{2}$$

where $f=1.4$.

1 At 0 °C, the thermal pressure coefficient $(\partial p/\partial T)_V=\alpha_p/\kappa_T$ is ~-1.3 bar/deg, where α_p is the thermal expansivity and κ_T is the compressibility of the aqueous suspension. (At 4 °C, $\alpha_p=0$; therefore $(\partial p/\partial T)_V=0$.)

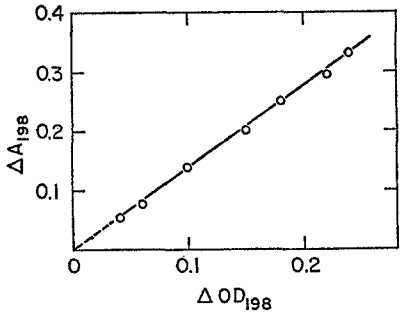

Fig. 3

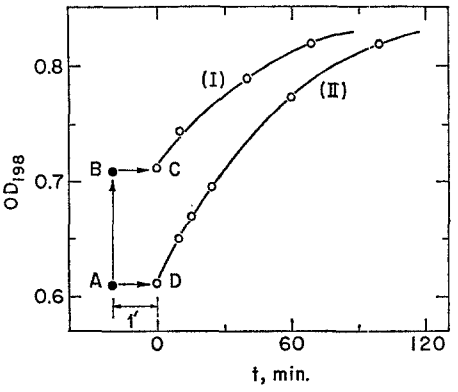

Fig. 4

Fig. 3. Correlation between ΔOD_{198} of the vesicle suspension and the corresponding change in the absorbance ΔA_{198} of the supernatant; *see* text

Fig. 4. Thermal release of vesicle suspensions OD_{198} as a function of time. At point A, an electric impulse ($E_0 \simeq 20$ kV/cm) is applied at 0 °C (arrow A to B); this sample and another nonpulsed one, are then brought from 0 °C to 23 °C within 1 min (arrows B to C and A to D, respectively). Curves I and II (o-o-o) are the result of subsequent thermal release at 23 °C (*see* text)

Provided the extinction coefficients ε_λ are given, the molar ratio of CA to ATP can be determined by fitting the measured absorbance spectrum A_λ to a linear combination of the absorbances of these two components:

$$A_\lambda^{1\,\mathrm{cm}} = \varepsilon_{\lambda,\,\mathrm{CA}}\, c_{\mathrm{CA}} + \varepsilon_{\lambda,\,\mathrm{ATP}}\, c_{\mathrm{ATP}} \tag{3}$$

where c is the molar concentration of CA and ATP, respectively. For the ratio $c_{\mathrm{CA}}/c_{\mathrm{ATP}} = 4:1$, the measured and the calculated spectra fit very well. In fact, a ratio of about 4:1 is found in the intact chromaffin granules (Smith, 1968). The absence of any additional absorbance in the range between 200 and 250 nm is a strong indication that no soluble protein was released. This is in accord with biochemical data (Hillarp, 1958) showing that in a temperature range between 20 and 40 °C only CA and ATP are thermally released from chromaffin granules.

When vesicle suspensions are stored at temperatures below 10 °C, no thermal release takes place under the given experimental conditions; no CA and ATP could be detected even after 10 hr.

Impulse Experiments

Effect of One Impulse. After applying an electric impulse at 0 °C to the vesicle suspension, an aliquot is transferred from the Teflon cell to a spectrophotometer cell and the spectrum of the suspension is recorded. For an

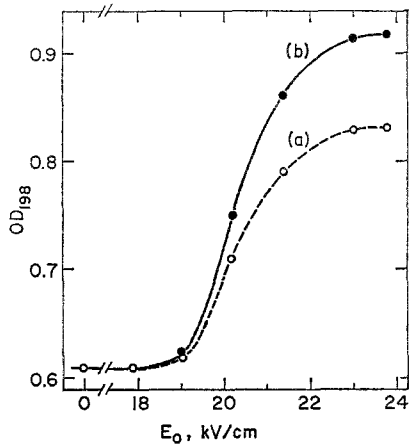

Fig. 5. OD_{198} as a function of the initial field strength E_0 of the impulse applied at 0 °C. (*a*) OD_{198} of the pulsed suspension, (*b*) $OD_{198} = A_{198}$ of the supernatant; *see* text

initial field intensity of 20.2 kV/cm, an increase in OD_{198} is obtained, as shown in Figs. 1 and 4.

When the optical density of the pulsed solution is followed at 23 °C as a function of time, one observes further thermal release. This suggests that only a fraction of the vesicular content has been liberated by an electric impulse of the initial field intensity of about 20 kV/cm. Furthermore, it is seen that the course of thermal release of the pulsed solution is identical with that of the nonpulsed one; compare curves I and II in Fig. 4. Both curves seem to approach the same saturation value.

In neither case is there release of soluble protein. When the vesicles from the experiment just described are separated by centrifugation and resuspended in distilled water, a further increase in OD caused by lysis is observed (Fig. 1). The spectra of the lysed vesicle suspensions are the same irrespective of whether the sample was pulsed or not. Lysis by osmotic shock also releases proteins from the vesicles.

Variations of the Impulses. It was observed that the extent of release depended on the initial field intensity of the impulse applied. Furthermore, there is a lower limiting field intensity E_0^m, for which ΔOD_{198} is within the limits of spectrophotometric recording noise (i.e., smaller than 0.005). Under our experimental conditions, $E_0^m \simeq 18$ kV/cm. For increasing values of E_0, ΔOD_{198} increases (as seen in Fig. 5) and reaches a saturation value, which is the same as that found in the thermal release of vesicle suspensions that were not pulsed. A suspension to which an impulse of the initial field intensity $E_0 \simeq 24$ kV/cm was applied, does not exhibit further efflux of CA

and ATP because of thermal release. The maximum release in this case is at least 40% of the total CA and ATP content of the vesicle preparation.

Whereas osmotic lysis also releases proteins from the vesicles, the electric impulses applied in our experiments do not lead to detectable protein liberation. If a second impulse of $E_0 \simeq 20$ kV/cm is applied to a vesicle suspension at 0 °C, it is observed that the ΔOD_{198} is about 60% of that caused by the first impulse. The amount of CA and ATP released per impulse decreases with increasing number of impulses.

Discussion

Release of part of the CA and ATP content of the isolated granules of the bovine medulla occurs readily at higher temperatures. Since at temperatures below 10 °C this thermal release is negligible, the impulse experiments were performed at 0 °C. Even at the maximum field intensity applied, the temperature increase accompanying the electric impulse is smaller than 6 °C. We can therefore assume that the impulse-induced release of CA and ATP is not thermally caused. Furthermore, the relatively large rise-time of the temperature increase excludes that the release is caused by transient local pressure increase in the suspension.

In the previous section it was shown that an electric impulse of proper strength and duration releases only a *fraction* of the vesicular content. It is very unlikely that this fractional release results from inhomogeneity of the electric field. The Teflon cell used for the impulse experiments is constructed so as to provide a homogeneous field density between the two electrodes (*see* Fig. 2). It is also unlikely that field intensities and impulse duration in these experiments can lead to gross mechanical distortion of whole vesicles. Consequently, we may assume that partial release is not caused by total efflux (similar to osmotic lysis) from a fraction of vesicles, whereas the remaining portion is not affected by the impulse. This assumption is supported by the fact that no protein could be detected, even when several impulses were applied to the suspension. A field intensity of 25 kV/cm corresponds to a potential difference of 25 mV per 100 Å. A transient potential difference of this strength, superimposed on the intrinsic membrane field, is very unlikely to cause membrane damage (dielectric breakdown).

A comparison of Figs. 4 and 5 shows that the efflux reaches an upper limiting value in both the thermal release and in impulse experiments. The effects of thermal motion and electric fields, up to about 25 kV/cm, are thus apparently "comparable": the permeability of the vesicle membrane to CA and ATP is enhanced by temperature increase as well as by electric impulses.

Based on the considerations outlined above, we assume that the electrically induced transient permeability change is caused by direct action of the electric impulse on the charges and/or dipoles associated with the membrane constituents.

Surface Charge Density

Electrophoretic experiments have shown that bovine chromaffin granules have a net negative surface charge at neutral pH. These polyanionic vesicles are therefore surrounded by a diffuse layer of ions forming an ionic atmosphere. The counterions of this cloud partially screen the repulsive forces between the negatively charged groups on the vesicle surface.

The number of the excess surface charges can be determined from the electrophoretic mobility u of the vesicles. Recent results of Banks (1966) are used to estimate an average value $\bar{u}=6\times10^{-5}$ cm^2/V sec for granules migrating electrophoretically at 4°C in 0.27 M sucrose-0.15 M KCl solution.

The vesicle surface charge density σ is calculated using the Gouy-Chapman equation:

$$\sigma=\left(\frac{2N\varepsilon kT}{10^3\pi}\Gamma\right)^{1/2}\sinh\frac{e\zeta}{2kT}. \tag{4}$$

$N=6\times10^{23}$ is Avogadro's number, $k=1.38\times10^{-16}$ erg/deg is the Boltzmann constant, $e=4.8\times10^{-10}$ stat C is the elementary charge, T is the absolute temperature, and Γ is the ionic strength of the medium. The dielectric constant ε of the solution near the vesicle surface is assumed to be that of (bulk) water. The zeta potential (ζ) is defined as the potential difference between the fixed surface charges and the mobile ion layer.

The thickness of the ion cloud surrounding the vesicle is characterized by the Debye-Hückel parameter κ:

$$\kappa^2=8\pi e^2N\Gamma/(10^3\varepsilon kT). \tag{5}$$

The conditions of the electrophoresis experiments were $T=277$ °K, $\varepsilon=85.7$ and $c=0.15$ M KCl. In dilute suspension, Γ is mainly determined by the excess uni-univalent electrolyte. Thus, $\Gamma=c=0.15$ M, and therefore $\kappa=1.28\times10^7$ cm^{-1}.

Electron micrographs of Helle, Flatmark, Serck-Hanssen and Lönning (1971) suggest an average value of the vesicle radius $\bar{a}$ of about 1.2×10^{-5} cm. Thus $\kappa\bar{a}=153.5$. The theoretical treatment of Wiersema, Loeb and Overbeek (1966) shows that for $\bar{a}\kappa\simeq200$ and for $\zeta<25$ mV, we can calculate the ζ-potential fairly accurately, using the von Smoluchowski relation

$$\zeta=4\pi\eta u/\varepsilon \tag{6}$$

where η is the viscosity coefficient of the medium. Here $\eta(4\ ^\circ C) \simeq 2.5 \times 10^{-2}$ g/cm sec. With $u = \bar{u} \simeq 6 \times 10^{-5}$ cm^2/V sec, we obtain $\zeta \simeq 19.8$ mV. Inserting in Eq. (4) we obtain $\sigma \simeq 5.8 \times 10^3$ stat C/cm^2. Dividing by the elementary charge, we find that the vesicle surface has 1.2×10^{13} elementary charges per cm^2. Hence, the average distance between the monovalent charge centers is $\bar{d} = 28.8$ Å.

The experimental conditions of the impulse experiments are $T = 273\ ^\circ K$ and $c = 0.01$ M NaCl. With the aid of Eq. (5) we obtain for the Debye-Hückel radius: $1/\kappa = 30.8$ Å. We may now assume that the chromaffin granules of our preparation have the same surface charge as those used in the electrophoretic experiments of Banks (1966). Comparing the values of $\bar{d}$ and $1/\kappa$ we conclude that the ionic atmospheres around the charge centers on the vesicle surface overlap, thus forming a continuous ion cloud of periodically varying ion density.

Polarization Mechanism

At present, no theoretical treatment is available for the quantitative analysis of the electrically induced release of material from vesicles. The apparent saturation level of release corresponds most likely to equal activities of the permeants within the vesicle core and the suspending medium. The existence of a threshhold for E_0 and the shape of the OD *vs.* E_0 curve (*see* Fig. 5) indicate that the release is not controlled by a simple reaction linearly dependent on the field intensity. It is therefore assumed that electric polarization may contribute to the release mechanism. The free energy change ΔG_p caused by induced polarization is proportional to E_0^2, for small field intensities. If in the sequence of events leading to release, there is a process depending linearly on ΔG_p, we expect that the induced OD change is correlated with a term proportional to E_0^2. (*See* the "low field" range of the OD *vs.* E_0 curve in Fig. 5.)

Electric impulses of the same order of magnitude as those used here were found to induce conformational transitions in polynucleotide complexes. In such systems the electric impulse is capable of shifting the ionic atmosphere relative to the associated polyanions. The displacement of the screening counterions causes increased electrostatic repulsion between the polyanions and leads to partial complex dissociation (Neumann & Katchalsky, 1972).

It is now intriguing to consider whether a polarization mechanism involving the ionic atmosphere can contribute to the electric induction of permeability changes of the vesicle membrane. Because of the relatively large surface charge density of the vesicle membrane, the average density

of ions in the ion cloud is higher than the ion density of the surrounding medium. A strong electric field can therefore cause a displacement of the ion cloud (relative to the vesicle) which is not completely compensated by an ion influx from the bulk toward the vesicle surface. An electric impulse capable of shifting the ionic atmosphere will, therefore, transiently polarize the vesicular system. Consequently, near the "negative pole" of the induced macro-dipole the counterion screening of a part of the surface charge is reduced. The resulting increase in the electrostatic repulsion of the ionic groups will, in turn, alter the energetically balanced structural arrangement of the membrane constituents. The very fact that thermal release occurs at temperatures higher than 10 °C indicates that the barriers preventing efflux of vesicular content at 0 °C are relatively low. It is therefore possible that minor changes in the relative position of charged groups and dipoles on the membrane surface lead to appreciable permeability changes.

We may now consider the processes necessarily involved in a mechanism in which counterion polarization contributes to the induction of permeability changes in vesicle membranes. According to this concept, the polarization of the vesicle should be intensive and last a sufficiently long time so as to permit structural rearrangements of the membrane and subsequent permeation of CA and ATP (as a complex or as single ions).

The strength of the polarization (induced dipole moment) depends on the intensity of the external field. In our case, the field intensity decays during an impulse. The time interval of strong polarization, caused in the range of the high fields of an impulse (Fig. 2*b*), is therefore dependent on the response time of the counterion atmosphere to the electric impulse; effective polarization requires a small relaxation time of the ion cloud. The theory of Schwarz (1962) provides a simple expression for the average relaxation time $\bar{\tau}$ of the counterion polarization of spherical particles,

$$\bar{\tau} = r^2/(2wkT) \tag{7}$$

where r is the sphere radius and w is the (mechanical) mobility of the counterions in the ionic atmosphere. For the chromaffin vesicles $r = \bar{a} = 1.2 \times 10^{-5}$ cm; we assume that $w = w(\mathrm{Na}^+, 0.01\ \mathrm{M}, 0\ ^\circ\mathrm{C}) \simeq 6 \times 10^8$ cm/sec dyne. Thus $\bar{\tau} = 31.6$ μsec.

The establishment of the polarization is thus much faster than the field decay time $\tau_E = 150$ μsec. Thus, already in the high field range of the impulse, the vesicle becomes polarized.

In the time interval of strong polarization, the permeability change could be based on lateral displacements of membrane components such as the lipids. The displacement times of lipids in the lipid bilayer are smaller

than 0.1 msec (Kornberg & McConnell, 1971). If we ascribe an average permeability of about 10^{-3} cm/sec to the permeants CA and ATP, corresponding to a permeation coefficient of about 10^{-9} cm^2/sec (Stein, 1967), the permeation time across a membrane of 60 Å thickness is about 0.6 msec. In this very rough estimate we obtain a time interval of about 0.7 msec during which the vesicle is "effectively" polarized. The lower limiting initial field intensity permitting release was found to be about 18 kV/cm. For this case, the electric field has decayed after 0.7 msec to about 0.2 kV/cm. This is the roughly estimated lower limiting field strength at which permeation can occur.

The changes in membrane potential necessary to depolarize nerves as well as chromaffin cells are known to be of the order of 20 mV. Assuming a membrane thickness of about 100 Å, this potential change corresponds to a change of about 20 kV/cm in the electric field intensity, in the range of values at which release was observed in the present experiments. This observation suggests that an event similar to the one studied here, may take place during neurosecretion. An assessment of its biological significance requires further experimental study. Obviously, there are many features to the neurosecretory process in the intact organ, which the present system, because of its simplicity, cannot attempt to reproduce. So, for instance, it was mentioned before that in the adrenal medulla, *all* soluble components are released in amounts corresponding to their relative abundance in the intact storage vesicles. This is the strongest indication so far that secretion in the adrenal medulla involves an exocytotic step. Such a step would evidently require that during the electric impulse the vesicles are in contact with the cell membrane, possibly with the aid of bridging Ca^{++} ions – a feature which is not a part of our experimental conditions. Incidentally, quantitative measurements of release at the adrenergic synapse indicate that the ratio of proteins to NE released is only about 1/10 to 1/100 that of the ratio in the intact adrenergic vesicles. Similarly, in electrophysiologic experiments the time interval between the depolarization of the presynaptic terminal and the appearance of neuroamine in the synapse may be significantly larger than the very short times during which field-induced release from vesicles occurs. Here again factors pertaining to other components of the secretory apparatus may be operative. In summary, the possibility has to be taken into account that electric field-induced permeability changes in vesicle membranes, leading to release of biogenic amines, may be a part of the complex chain of processes constituting neurohumoral secretion.

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References

Banks, P. 1966. An interaction between chromaffin granules and calcium ions. *Biochem. J.* **101**:18C.

Bloom, F. E., Iversen, L. L., Schmitt, F. O. 1970. Macromolecules in synaptic function. *Neurosci. Res. Prog. Bull.* **8**:325.

Duysens, L. N. M. 1956. The flattening of the absorption spectrum of suspensions, as compared to that of solutions. *Biochim. Biophys. Acta* **19**:1.

Eigen, M., De Maeyer, L. 1963. Relaxation methods. *In*· Technique of Organic Chemistry. S. L. Friess, E. S. Lewis, and A. Weissberger, editors. Vol. 8, Pt. 2, p. 895. Interscience Publishers, Inc., New York.

Euler, U. S. von. 1970. Adrenergic neurotransmitter functions. *Science* **173**:202.

Helle, K. B., Flatmark, T., Serck-Hanssen, G., Lönning, S. 1971. An improved method for the large-scale isolation of chromaffin granules from bovine adrenal medulla. *Biochim. Biophys. Acta* **226**:1.

Hillarp, N. A. 1958. The release of catecholamines from the amine containing granules of the adrenal medulla. *Acta Physiol. Scand.* **43**:292.

Kornberg, R. D., McConnell, H. M. 1971. Lateral diffusion of phospholipids in a vesicle membrane. *Proc. Nat. Acad. Sci.* **68**:2564.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L., Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265.

Neumann, E., Katchalsky, A. 1972. Long-lived conformation changes induced by electric impulses in biopolymers. *Proc. Nat. Acad. Sci.* **69**:993.

Schwarz, G. 1962. A theory of the low-frequency dielectric dispersion of colloidal particles in electrolyte solution. *J. Phys. Chem.* **66**:2636.

Smith, A. D. 1968. Biochemistry of adrenal chromaffin granules. *In*: The Interaction of Drugs and Subcellular Components in Animal Cells. P. N. Campbell, editor. p. 239. J. & A. Churchill, Ltd., London.

Stein, W. D. 1967. The Movements of Molecules across Cell Membranes. p. 36. Academic Press Inc., New York.

Trifaró, J. M., Dworkind, J. 1970. A new and simple method for isolation of adrenal chromaffin granules by means of an isotonic density gradient. *Analyt. Biochem.* **34**:403.

Wiersema, P. H., Loeb, A. L., Overbeek, J. T. G. 1966. Calculation of the electrophoretic mobility of a spherical colloid particle. *J. Colloid Interface Sci.* **22**:78.