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POTASSIUM ION-INDUCED SWELLING OF NERVE-ENDING PARTICLES BY LIGHT-SCATTERING MEASUREMENT

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SUMMARY

The osmotic behavior of nerve-ending particles isolated from rat brain cortex was studied by light-scattering measurements. When suspended in sucrose or saline solutions of various osmolarities, the light-scattering properties of nerve-ending particles were not so simple as those of the brain microsomes reported earlier. But it was confirmed that changes in the scattering at 45° (I_{45}) reflected volume changes of nerve-ending particles in sucrose or saline solutions.

With the measurement of I_{45}, it was demonstrated that the nerve-ending particles in sucrose, NaCl, and LiCl of various osmolarities behaved as osmometers obeying the simple Boyle–Van 't Hoff's relation. But, in the presence of K^+, they showed a marked deviation from the relation. Under a fixed total osmolarity of NaCl and KCl, K^+ exceeding about 30 mM induced the swelling of nerve-ending particles, such an increase in the particulate volume being proportional to logarithm of K^+ concentration. Replacing K^+ by Rb^+ or Cs^+, a similar effect was observed. It was also demonstrated that treatment of nerve-ending particles with various chelating agents such as EDTA prompted the K^+-induced swelling of nerve-ending particles, the greater the effect, the larger the stability constant for Ca^{2+} of a chelating agent applied, whereas some inhibitors such as ouabain and *N*-ethylmaleimide scarcely affected the K^+-induced swelling.

INTRODUCTION

In studies on size, shape and structure changes of isolated subcellular particles such as mitochondria and microsomes, the measurement of light-scattering has been frequently employed: for instance the method has been applied to chloroplast grana[1,2], to mitochondria[3,4] and to mitochondrial fragments[5]. In our previous report we also applied angular light-scattering measurements to brain microsomes to study their osmotic behavior[6] and vesicular shrinkage caused by ATP[7] or NAD(P)H[8]. When light-scattering measurements are applied to particles far larger than microsomes, however, it is expected that our method is no longer straightforwardly applicable for estimating the volume changes. Indeed, trying to extend our method to nerve-ending particles isolated from rat brain, it was found that light-scattering data on nerve-ending particle suspensions could not be treated as simply as those on microsomes. It is well known that the boundary membrane of pinched-off nerve-ending is resealed

so that nerve-ending particles swell, passing into a so-called ghost when subjected to hypoosmotic shock[9]. Nevertheless, changes in light-scattering of nerve-ending particles caused by hyper- and hypotonic sucrose, NaCl and KCl apparently did not always conform to each other. During the course of these experiments, it was found that swelling of nerve-ending particles was induced in KCl media, which resulted in a discrepancy in light-scattering. This article describes a method of estimating relative volume changes of nerve-ending particles with light-scattering measurements and some properties of the K^+-induced swelling of the nerve-ending particles.

METHODS

Rats of both sexes weighing 200–250 g were killed by decapitation and the whole brains were removed immediately into ice-cold 0.32 M sucrose containing 20 mM Tris–HCl buffer (pH 7.3) and rinsed. The brain cortex was separated by a spatula and pooled cortical tissues (usually 2–3 brains) were homogenized in 10 vol. of 0.32 M sucrose by several strokes of a Teflon–glass homogenizer of Potter–Elvehjem type (clearance about 0.2 mm).

Using a Hitachi preparative ultracentrifuge (Type HU 40P), the nerve-ending particles were prepared by density gradient centrifugation according to the procedure of Gray and Whittaker[10], with a slight modification: the microsomal supernatant was separated by centrifugation at 12000 × *g* for 20 min and the crude mitochondrial fraction thus obtained was subjected to the density gradient centrifugation at 64000 × *g* for 60 min instead of the original 53000 × *g* for 2 h.

The material at the 0.8–1.2 M interface of discontinuous density gradients was carefully removed using a capillary with a fine tip and diluted with buffered 0.2 M sucrose and spun down at 12000 × *g* for 20 min. The resultant pellets were resuspended in buffered 0.32 M sucrose and spun down at 12000 × *g* for 20 min. Such a washing procedure with resuspension and centrifugation was repeated twice and then the final pellets were suspended in buffered 0.32 M sucrose (about 1 ml per g brain cortex tissues) to form the stock suspension, which was stored at 0 °C. When salt solution was used as a suspension medium, the final pellets were suspended in appropriate buffered salt solutions after washing twice with the buffered salt solution (pH 7.3) as described above.

The stock suspension was submitted to light-scattering measurements after dilution with a Tris-containing sucrose or saline of the appropriate concentration or distilled water to obtain the desired osmolarity and/or a suitable particulate concentration. When Na^+ in the suspending media was partially replaced with K^+, Rb^+ or Cs^+, a stock saline suspension of high particulate concentration was appropriately diluted with a solution of the chloride salt of these ions.

Light-scattering measurements were made with a Brice–Phoenix type photometer (Shimadzu PG 2 type), measurements of the refractive index for λ of 589 nm were made with an Abbe refractometer (Shimadzu Bausch and Lomb, Type 3L), while protein assay and determination of the water content of the nerve-ending particle were carried out by essentially the same procedures as those employed in our previous work on brain microsomes[6]. The refractive index, μ_1, for $\lambda = 368$, 436, 546 and 578 nm was calculated using Cauchy's dispersion formula.

Just as with the microsomal suspensions, the intensity of light scattered at the

angle 45° or 90° (I_θ at $\theta=45°$ or 90°) from nerve-ending particle suspensions of a given osmolarity changed in parallel with the particulate concentration (n) in the range from 20 to 100 μg protein/ml, irrespective of whether the suspending media used was sucrose or saline. It follows that the ratio of I_θ in any osmolarity to that in the isotonic medium (0.32 osM), $I_\theta{}^i$, is independent on n. On the other hand, the smaller the θ and λ, the higher the value of I_θ. Therefore, I_{45} with a λ of 368 nm was routinely employed to estimate volume changes of nerve-ending particles, I_{45} being usually normalized to I^i_{45}.

On changing the osmolarity of the medium, the value of I_{45} of nerve-ending particles reached a nearly constant level within several minutes and remained at this level for at least 1 h so that the scattering measurements were usually carried out 30 min after incubation of nerve-ending particles at room temperature (20–22 °C).

RESULTS

The dependence of I_{45} of nerve-ending particle suspension on the osmolarity of suspending media

When nerve-ending particles were suspended in sucrose media, I_{45} from the particulate suspensions varied linearly with the reciprocal of the sucrose concentration, $1/c$, at least in the range of $c=0.16$–0.64 osM. As shown in Fig. 1A, such a linear relationship between I_{45} and $1/c$ holds for any wavelength used and the lines with different λ converge at a point on the abscissa where $I_{45}=0$ and $c=c^e$, c^e, being negative, *i.e.* an imaginary "negative" concentration. It follows that I_{45} is given by

$$\left.\begin{aligned} & I_{45}/I^i_{45} = (1/c - 1/c^i)/(1/c^i - 1/c^e) \\ \text{or} \quad & \Delta I_{45} = \frac{I_{45} - I^i_{45}}{I_{45}{}^i} = \gamma \cdot \left(\frac{c^i}{c} - 1\right) \end{aligned}\right\} \qquad (1)$$

where the superscript i denotes the quantities at the isotonic medium and $\gamma=(1-c^i/c^e)^{-1}$. Independent measurements on different samples showed that the value of c^e and so of γ could be regarded as a constant; $\gamma=0.401\pm0.034$ ($n=6$).

Just as in sucrose media, I_{45} from nerve-ending particles observed in NaCl media also varied linearly with the reciprocal of the osmolar concentration regardless of the λ used and the family of straight lines standing for each λ converges at a point on the abscissae (Fig. 1B). It is obvious, therefore, that the relationships in Eqn 1 also hold for NaCl media. But a definite difference between both media is obvious; the lines for NaCl fall but those for sucrose rise as $1/c$ increases, and so c^e is positive for NaCl but negative for sucrose. In NaCl media, γ is negative because $c^e > c^i$ and $\gamma=0.221\pm0.014$ ($n=6$).

Such a difference in light-scattering properties between sucrose and saline media has already been noted with the extinction measurement ($\lambda=520$ nm) by Keen and White[11]. These investigators ascribed such a difference to the difference in μ_1 between both media. The μ_1 of the sucrose solution is not so very different from that of NaCl solutions so that their explanation seems to be not so convincing. Whatever the theoretical background for such an opposite scattering effect observed in sucrose

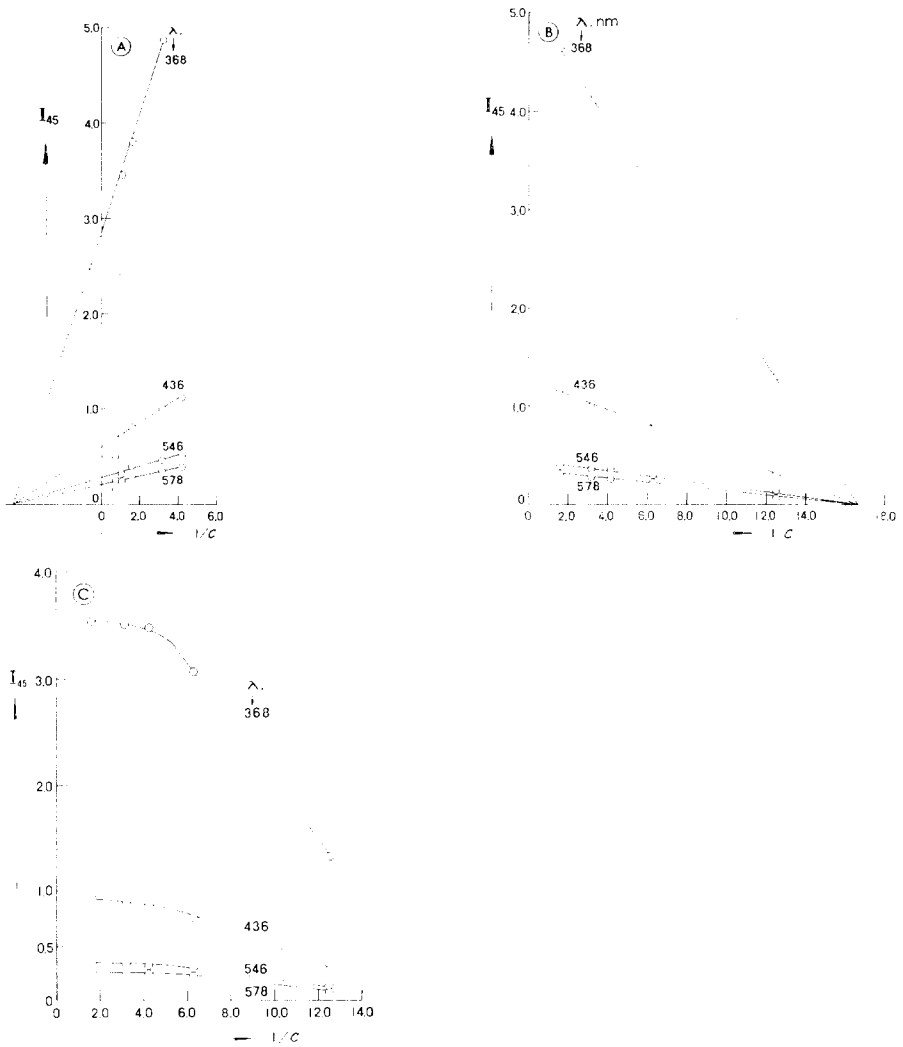

Fig. 1. The dependence of light-scattering at 45° (I_{45} normalized to that at 436 nm in 0.32 osM sucrose (A) or in 0.32 osM NaCl solution (B and C)) of a nerve-ending particles suspension (42.3 μg protein per ml) on the reciprocal of osmolar concentration ($1/c$). (A) in sucrose solution; (B) in NaCl solution; (C) in KCl solution.

and saline may be, the present experiment confirmed the results reported by these investigators.

At a fixed concentration, the μ_1 of LiCl or KCl solutions is almost identical with that of NaCl. It is expected, therefore, that I_{45} *vs* $1/c$ relation in these solutions is identical with each other. Our measurements showed that it was the case for LiCl but not so for KCl as shown in Fig. 1C, in which I_{45} in KCl media was normalized to I_{45}^1 of NaCl to make comparison easy. I_{45} in hyperosmotic KCl media is significantly lower than that in NaCl media in spite of an almost identical c^e and so the linearity in the I_{45} *vs* $1/c$ relationship is lost. Such a finding cannot be explained

by the effect of μ_1 and appears to indicate the inapplicability of Eqn 1 to KCl media. This fact is the starting point of our studying K^+-induced swelling of nerve-ending particles.

Relationship between changes in I_{45} and the particulate volume

In spite of the difference in the slope between sucrose and NaCl media, the linear I_{45} *vs* $1/c$ relation, Eqn 1, suggests that the osmotic volume changes of nerve-ending particles in both media are reflected in changes in the I_{45} observed. The Boyle–Van 't Hoff's law is usually written as

$$\left.\begin{aligned} &c(v-v_d)=c^i(1-v_d)=k \\ \text{or}\quad &\\ &\Delta v=(v-1)=\left(\frac{c^i}{c}-1\right)v_f, \qquad v_f=1-v_d \end{aligned}\right\} \tag{2}$$

where v is the relative mean volume of nerve-ending particles referred to that in isotonic media (v_i), v_d the so-called osmotic dead space expressed as a fraction of v_i and k is a constant. It follows from Eqns 1 and 2 that

$$\Delta I_{45}=\Delta v/\gamma \cdot v_f \tag{3}$$

As stated above, γ is constant, whereas v_d and so v_f can be regarded as constant. Indeed, as shown in Table I, the determination of the water content proves, in spite of its limited accuracy, that the relation 2 applies to osmotic behaviors of nerve-ending particles in both media, v_d being about 0.64 and so $v_f=0.36$. Using the value of γ given above, Δv can be estimated from Eqn 3. The results are summarized in Fig. 2.

Using the linear relationship between ΔI_{45} and Δv in NaCl media as the calibration curve, volume changes of nerve-ending particles in KCl media were also evaluated, while their water content was measured as another series of the experiments. As seen in Fig. 2, both results agree fairly well with each other. Such a fact indicates that the relation (3) also applies to KCl media, the difference in I_{45} *vs* $1/c$ relation between NaCl and KCl media (Figs 1B and 1C) being only due to the swelling of nerve-ending

TABLE I

OSMOTICALLY-INDUCED CHANGES IN THE WATER CONTENT OF NERVE-ENDING PARTICLES AND THEIR OSMOTIC DEAD SPACE

c^i: 320 mosM; c: osmolarity (mosM) of medium; v_d: osmotic dead space. Means ± S.E. are given.

Osmolarity (c) (*mosM*)	*Medium*	*Water content* (*mg/mg dry pellet weight*)	*Relative water content* (v)	Δv	$\Delta v/(c^i/c-1)$	v_d
161.8	NaCl	9.28 ± 0.21 ($n=3$)	1.34 ± 0.06	0.34 ± 0.05	0.35 ± 0.05	0.65 ± 0.05
160.0	Sucrose	9.09 ± 0.24 ($n=5$)	1.33 ± 0.03	0.33 ± 0.03	0.33 ± 0.03	0.67 ± 0.03
320.0	NaCl	6.92 ± 0.46 ($n=4$)	1.0	0	—	—
320.0	Sucrose	6.83 ± 0.31 ($n=4$)	1.0	0	—	—
583.4	NaCl	5.81 ± 0.37 ($n=4$)	0.84 ± 0.05	−0.16 ± 0.05	0.36 ± 0.11	0.64 ± 0.11

particles in KCl media. Thus it is obvious that the light-scattering measurement was applicable to the estimation of the K^+-induced swelling of nerve-ending particles.

The measurements of I_{45} have a great advantage over the measurement of water content; the amount of a nerve-ending particle sample needed is far smaller (1:1000 or less) and the measurement far less time-consuming. Applying the relation 3, therefore, volume changes of nerve-ending particles in saline media were followed using this method.

The dissymmetry coefficient and particulate volume

As reported in our previous paper[6], the measurement of $[Z]$, the value of the dissymmetry coefficient obtained by extrapolating the particulate concentration to zero, was successfully applied to the evaluation of osmotic volume changes of brain microsomes. In the present study, however, the $[Z]$ of nerve-ending particles was found not to be simply related to v so that the following procedure was tried. The $[Z]$ of spherical particles (diameter D and refractive index μ_2) chiefly depends on $\alpha = \pi D/\lambda'$ and so $[Z] \propto v/\lambda'$ under a constant effective refractive index, m ($=\mu_2/\mu_1$). Since the Relation 2 was applicable to nerve-ending particles in sucrose and saline media, $[Z] \propto v$ is approximated by

$$v^{1/3} = (1+\Delta v)^{1/3} \doteqdot 1 + \Delta v/3 \propto \frac{1}{c} + \frac{3-v_i}{c^i v_i}$$

and so

$$\alpha \propto \frac{1}{c} + \frac{2+v_d}{c^i(1-v_d)}$$

Since $c^i = 0.32$ and $v_d \doteqdot 0.64$, the last term is approx. 22. We tried, therefore, to plot $[Z]$'s observed in various osmolarities against $(1/c+22)/\lambda'$. As shown in Fig. 3, such

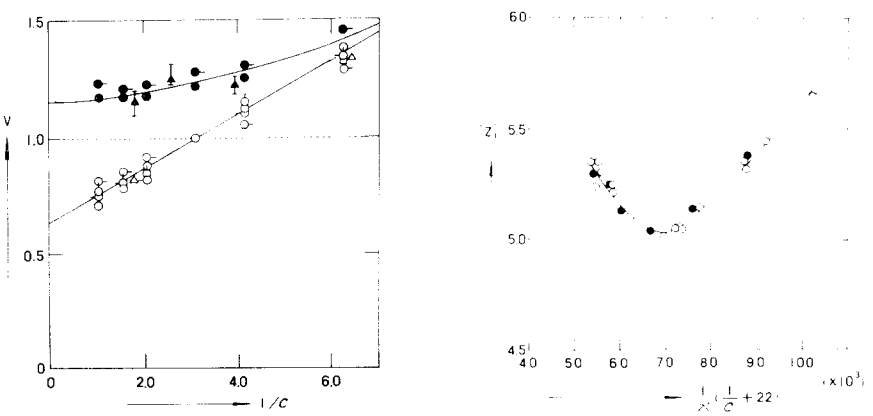

Fig. 2. The relationship between the reciprocal of an osmolar concentration of the suspension media (sucrose (○, □), NaCl (○, ○-, △), LiCl (-○) and KCl (●, ●-, ▲)) and the relative volume of nerve-ending particles. ○, ○-, ●-, estimated from $[Z]$; -○, ○, ●, estimated from I_{45}; △, ▲, □, data based on the measurement of water content. Relative volume (v), normalized to that in 0.32 osM NaCl solution.

Fig. 3. Relationship between the dissymmetry coefficient ($[Z]$) of nerve-ending particles and $1/\lambda'(1/c+22)$ (see text). λ', wavelengths of incident light; c, osmolar concentration. ○, in sucrose; ●, in NaCl.

a plot results in an almost single curve convex downwards. A slight shift towards the left of the points for NaCl media appears to be attributable to the slight difference in m, the effect of the difference in μ_1 on I_{45} and I_{135} being largely cancelled out by taking their ratio at the infinite dilution, $[Z]$. At any rate, if small errors in results are of no consequence, the curve might be regarded as single regardless of whether the suspending media is sucrose or saline.

The figure clearly shows that $[Z]$ is not single-valued with respect to c and so to α, a sharp contrast with the $[Z]$ *vs* α relationship observed on brain microsomes. Theoretically speaking, therefore, the determination of $[Z]$ alone could not afford a means to estimate volume changes of nerve-ending particles. As shown in the previous section, however, Eqn 2 applies to nerve-ending particles in sucrose and saline media, so that the use of the curve shown in Fig. 3 as the calibration curve enables us to correlate observed $[Z]$ values with $1/v$. Those observed in KCl media give c of NaCl osmotically equivalent to the swelling induced by K^+ so that $1/v$ is easily read off. The results thus estimated are also included in Fig. 2, which also show a fairly good agreement with those estimated from the measurements of I_{45} or of the water content.

In sucrose–NaCl or sucrose–KCl mixture, the measurement of I_{45} is not always straightforwardly applicable to evaluate changes in particulate volume, because the change in μ_1 seriously affects the Van 't Hoff's relation (Eqn 2) between I_{45} and v (see Fig. 1). In this respect, the $[Z]$ determination has great advantages; the effect of μ_1 is safely neglected if small errors in results are of no consequence. Thus the measurements of $[Z]$ of nerve-ending particles suspended in sucrose–NaCl, sucrose–KCl and the NaCl–KCl mixture of 320 mosM were tried by using the curve in Fig. 3 as the calibration curve. As shown in Fig. 4, the value of v remains practically unaltered in sucrose–NaCl media and is independent of the NaCl to sucrose ratio, while v increases in sucrose–KCl and NaCl–KCl media as the external concentration of K^+, $[K]_0$, increases. It might be said, therefore, that K^+-induced swelling of nerve-ending particles is practically independent of the presence of Na^+.

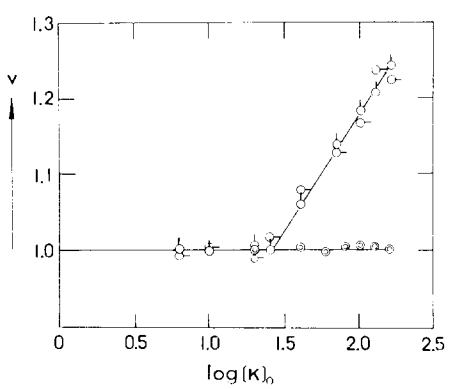

Fig. 4. K^+-induced swelling of nerve-ending particles in isotonic mixtures of sucrose–KCl (•) and NaCl–KCl (○). Abscissae, logarithm of K^+ concentration (mM). Ordinates, relative volume of nerve-ending particles (v) calculated from the I_{45} measurement at 436 nm normalized to that in the 0.32 M NaCl solution. Changes of v in the isotonic sucrose–NaCl (◎) mixture are also included as a comparison, for which the abscissae should be read as Na^+ concentration.

Dependence upon K^+ concentration of nerve-ending particle swelling

At a given salt concentration, the refractive index (μ_1) of NaCl solution is nearly identical with that of KCl and so of the NaCl–KCl mixture. Therefore, from Relation 3, changes in v of the nerve-ending particles suspended in NaCl–KCl mixtures of various osmolarities could be measured at various $[K]_0:[Na]_0$ ratios. When the total osmolarity of the saline mixture is fixed, the value of v remains nearly unaltered unless the $[K]_0$ exceeds a certain limit, $[K]_0^* \simeq 30$–40 mM, while v increases almost linearly with $\log[K]_0$ for $[K]_0 > [K]_0^*$ (Fig. 5A). If v at $[K]_0 < [K]_0^*$ is v^*, then v at given osmolarity is given by an approximate expression,

$$v - v^* = \alpha'(\log[K]_0 - \beta'), \qquad \text{for } [K]_0 \geqq [K]_0^* \tag{4}$$

where α' and β' are constants. As seen in the figure, α depends on the total osmolarity π, whereas β' is almost independent of π, corresponding to $\log[K]_0^*$. When the linear part of the $v - \log[K]_0$ relation for $[K]_0 > [K]_0^*$ is extrapolated below $[K]_0^*$, all lines appeared to converge, irrespective of π, to the point, P, corresponding to $v = 0.64 \simeq v_d$, the osmotic dead space, and $[K]_0 \simeq 4$ mM.

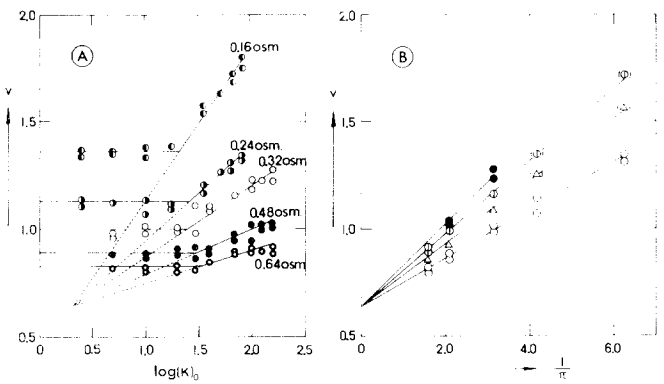

Fig. 5. Effects of K^+ concentration on the relative particulate volume (v) in NaCl–KCl media of various total osmolarities. (A) Ordinates, the particulate volume (v) referred to that in the isotonic NaCl medium; abscissae, logarithm of K^+ concentration (mM) in NaCl–KCl mixtures. (B) Ordinates, the same as in A; abscissae, reciprocal of osmotical concentration (osM). ○, KCl = 10 mM; △, KCl = 60 mM; □, KCl = 70 mM; ●, KCl = 170 mM. The plots given in parentheses are those obtained by interpolation in (A).

As seen in Fig. 2, Van 't Hoff's relation is expected to apply to v^*:

$$\pi(v^* - v_d) = k, \qquad \text{for } [K]_0 = 0 \sim [K]_0^* \tag{5}$$

Indeed, its applicability is clearly demonstrated by replotting v against π^{-1} (Fig. 5B). It follows that Eqn 4 can be transformed as follows:

$$\pi(v - v_d) = k + \alpha(\log[K]_0 - \beta'), \qquad \text{for } [K]_0 > [K]_0^* \tag{6}$$

where $\alpha = \alpha' \cdot \pi$. As seen in Fig. 5B, when v at a given $[K]_0$ greater than $[K]_0^*$ is plotted against π^{-1}, an almost linear relationship converging to the same intercept (v_d) as that obtained with Eqn 5 is obtained. Such a finding means that α' changes in inverse

proportion to π and so α is practically independent of π. Indeed, Fig. 6 directly demonstrates that α' is really inversely proportional to π. Thus, Eqns 5 and 6 give a generalized Boyle–Van 't Hoff's relation in the presence of K^+, which causes swelling of nerve-ending particles. Of course, the independence of β' on π is only approximate; in Fig. 5A a tendency that the higher the osmolarity the greater the value of β' can be seen. Strictly speaking however, the relations between v and $\log[K]_0$ presented in Fig. 5A might be curvilinear, Eqns 5 and 6 being only the approximation to two straight lines; one is horizontal (*i.e.* corresponding to Eqn 5), the other is a rising one which passes by the unique point P (v_d, about 4 mM) with a gradient of α. It may be rather better, therefore, to use a new parameter, $\beta = \alpha \cdot \beta'$ as follows,

$$\pi(v - v_d) = k + \alpha_M \log[M]_0 - \beta \qquad (6')$$

Here M denotes K^+ or monovalent cations having a swelling effect similar to that of K^+ and β remains constant so far as the straight line given by Eqn 6′ passes by the point P, while α_M depends on the cation species (see the next section).

Effects of anion on the K^+-induced swelling

To examine the effect of anions on the K^+-induced swelling, Na^+–K^+ mixtures composed of their salts other than chloride were used, which included bromide, iodide, fluoride and sulfate. These anions showed, however, hardly any difference from chloride in producing the swelling.

Effect of other monovalent cations

When RbCl or CsCl was used instead of KCl, a nerve-ending particle swelling quite similar to that in KCl media also occurred. But the α-values of Rb^+ and Cs^+,

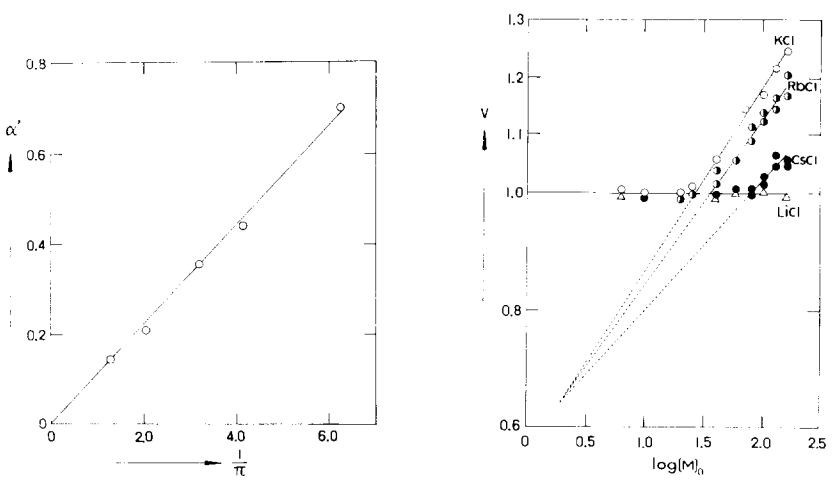

Fig. 6. An inversely proportional relationship between the slope constant, α' and the osmotic pressure, π. Ordinates, α' estimated from the plot shown in Fig. 2A. Abscissae, reciprocal of osmotic concentration (osM).

Fig. 7. Comparison of swelling of nerve-ending particles in isotonic KCl (○), RbCl (◑), CsCl (●) and LiCl (△). Ordinates, logarithm of concentration of KCl, RbCl, CsCl or LiCl.

$(\alpha)_{Rb}$ and $(\alpha)_{Cs}$, are smaller than that of K^+, $(\alpha)_K$, while the β'-value of the former two ions is greater than that of K^+. As an example, the results obtained with isotonic media are illustrated in Fig. 8, in which it is clearly seen that the extrapolated line of the linearly increasing part of both Rb^+ and Cs^+ also converges to one and the same dead space point, P, just as that of K^+, a fact indicating that Eqn 6′ remains the same for these three alkali metal ions in spite of their different α-values. In other words, the potency to cause nerve-ending particle swelling of these ions could be compared by the magnitude of α or by the approximate critical concentration $\beta'=\beta/\alpha$.

As stated above, nerve-ending particles in LiCl solutions behave just as in NaCl or sucrose media, to which a simple Boyle-Van 't Hoff's relation in the form given by Eqn 5 applies. We could also confirm this by using a NaCl-LiCl mixture of various tonicity. In this case, the α-value of Li^+, $(\alpha)_{Li}$, can be mathematically regarded as zero just as $(\alpha)_{Na}$. To summarize, the order of decreasing magnitude of α for the alkali metal ions tested is as follows:

$$(\alpha)_K > (\alpha)_{Rb} > (\alpha)_{Cs} > (\alpha)_{Na} = (\alpha)_{Li} = 0 \tag{7}$$

a series indicating that K^+ is the most favorable ion for nerve-ending particle swelling (Fig. 7).

Effects of some inhibitors on K^+-induced swelling

It was already demonstrated that the isolated nerve-ending particles were capable of actively incorporating a cation[12,13,14]. To examine the possibility of some contribution of the active mechanisms, the effects of some inhibitors were examined. These included cyanide (10^{-5} M), rotenone (2 mM), antimycin A (1 μg), amylobarbitone (2 mM), ouabain (10^{-3} M) and N-ethylmaleimide (NEM, $10^{-6} \sim 10^{-4}$ M). But the swelling of K^+ higher than 30 mM was found to be practically unaffected by the incubation with the inhibitors tested.

Effect of divalent cation

In view of the antagonism between Ca^{2+} and K^+ on physiological phenomena such as the membrane potential of nerve cells, we tried to examine the effects of Ca^{2+} and Mg^{2+} on the K^+-induced swelling by comparing the $v-\log[K]_0$ plot in the presence and absence of these divalent cations. Probably because of the particulate aggregation induced, however, the presence in suspending media of these ions even at a low concentration such as 0.4 mM was found to cause difficulty in obtaining scattering data which were reproducible and accurate enough for our purposes. On the other hand, such a trouble could be avoided by reducing their concentration to 0.2 mM or less, but the $v-\log[K]_0$ plot remained scarcely unaffected under such a condition.

Effect of some chelating agents

As stated above, the effects of Ca^{2+} and Mg^{2+} were difficult to study more in detail with the light-scattering technique. Instead of adding these ions into particulate suspensions, therefore, we examined the effects of some drugs chelating them. These included EDTA, glycoletherdiaminetetraacetate (GEDTA), 1,2-cyclohexanediaminetetraacetate (CyDTA) and nitrilotriacetate (NTA).

As an example, the results with EDTA in the isotonic media are presented in

Fig. 8, which clearly shows that this drug causes an increase in the slope constant, α, in Eqn 6', the higher the concentration the greater the increase in α, whereas the other constant, β, in Eqn 6' remains unaltered as seen by the effect with Rb^+ and Cs^+. Under hyper- and hypotonic conditions, the results were found to be same, while the simple Van 't Hoff's relation (Eqn 5) holds for K^+-free or low-K^+ media even in the presence of 1.0 mM EDTA. With GEDTA, CyDTA and NTA, the circumstances were quite similar, the only difference among them being that their concentration which was effective in causing a significant increase in α was different from each other.

The dose (X)–effect (Y) relation of these agents can be drawn by putting $Y=\{(\alpha_+)-(\alpha_-)\}/(\alpha_-)$, where (α_-) is the control value of α in the absence of a chelator, (α_+) the α-value in the presence of the chelator in an amount of X (mole per mg synaptosomal protein). As seen in Fig. 9, the Y–X plots for the agents tested are sigmoid

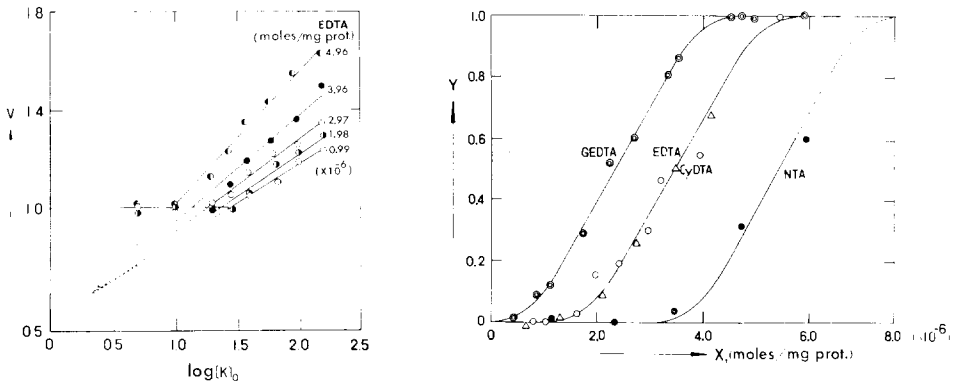

Fig. 8. Effects of EDTA on the $v-\log[K]_0$ relation in the isotonic media. Abscissae and ordinate, the same as in Fig. 2A.

Fig. 9. The dose-effect relationship of some chelating agents. ◎, glycoletherdiaminetetraacetate (GEDTA); ○, EDTA; △, 1,2-cyclohexanediaminetetraacetate (CyDTA); ●, nitrilotriacetate (NTA). Abscissae, concentration of chelating agents, X (moles/mg protein). Ordinate, $Y=[(\alpha_+)-(\alpha_-)]/(\alpha_-)$ (see text).

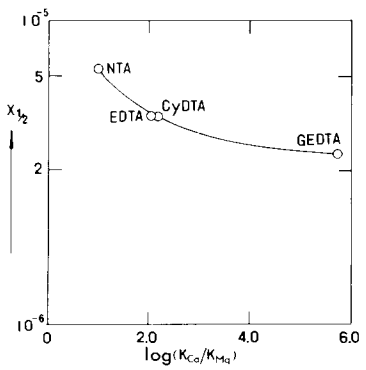

Fig. 10. Correlation between the Ca^{2+}-binding activity and half-maximal concentration, $X_{1/2}$ (moles/mg protein), of chelating agents obtained from Fig. 7. Abbreviations see legend to Fig. 9.

and nearly parallel. Now we can compare their potency of increasing α by comparing $(X)_{1/2}=X$ at Y corresponding to its half-maximal value. As can be seen from Fig. 10, $\log(X)_{1/2}$ varies log-linearly with K_{Ca}/K_{Mg}. Here, K_{Ca} and K_{Mg} are the stability constants for Ca^{2+} and Mg^{2+} of the chelating agents at pH 7.3 and their values reported by Danzuka and Ueno were used[15]. To summarize, the generalized Van 't Hoff's relation (Eqn 6') holds for nerve-ending particles even in the presence of Ca^{2+}-chelating agents, but the α-value increases in their presence, their potency being dependent chiefly, if not solely, upon their Ca^{2+}-binding power.

DISCUSSION

The results presented above demonstrate that changes in the mean volume of nerve-ending particles ($1/v$) in both sucrose and NaCl media are reflected in light-scattering changes, *i.e.* I_{45}, which linearly vary with the reciprocal of the osmolarity, $1/c$, as required by Boyle–Van 't Hoff's law. But correlation between both changes is not as simple as that of brain microsomes reported previously[6]. As seen in Figs 1A and 1B, osmotic shrinkage in saline causes an increase in I_{45} while the particulate shrinkage in hypertonic sucrose results in its decrease. Such findings are quite similar to the osmotically-induced changes in the extinction ($\lambda=520$ nm) of nerve-ending particles suspended in sucrose and NaCl solutions reported by Keen and White[11]. It follows that, with the measurement of I_{45} or the extinction alone, we cannot decide whether a decrease in the scattering means a decrease in the particle volume, other information, theoretical or empirical, is needed to decide this.

The results with the $[Z]$ measurement are quite similar to those with the I_{45} measurements; $[Z]$ of nerve-ending particles is double-valued with respect to c and $1/v$ can not be uniquely determined from a $[Z]$ value without other information concerning the osmotic behavior of the particles.

There is no satisfactory theory, however, available to treat quantitatively the light-scattering of complicated objects having the size, structure and the refractive index of subcellular particles such as nerve-ending particles. In the present study, therefore, the determination of the water content was used as a reference, from which $\partial I_{45}/\partial v$ is estimated by Eqn 4. Thus, the empirical nature of the light-scattering technique for estimating volume changes of nerve-ending particles is apparent. But it seems of great interest, as Keen and White[11] already pointed out, that the light-scattering technique can be available for quantitative studies on the permeability of nerve-ending particle membrane, which could be regarded as a model of the mammalian neuronal membrane.

Theoretically speaking, it is necessary, in order to relate I_{45} to the particulate volume changes, to measure I_{45} as a function of external osmolarity with μ_1 held constant. As discussed in detail previously[6], however, I_0 is relatively unaffected by such slight changes in μ_1, because the important scattering element is the particulate membrane which has a much greater μ than μ_1. Indeed, altering the value of μ_1 for NaCl (or sucrose) media by adding a small amount of sucrose (or NaCl) at a fixed c, confirmed that such a small change in μ_1 as that which resulted from the use of NaCl (or sucrose) alone (μ_1 is not fixed) did not seriously affect the $I_{45}-c$ relation (at most $\pm 5\%$ in I_{45}) in the range of $c=0.16$–0.64 osM. These facts provide a support for the

use of scattering measurements under unfixed μ_1, if small errors, within a few %, in the results are of no consequence.

Using such light-scattering techniques, it was found that K^+ induced swelling of nerve-ending particles (Fig. 2). The results presented in Fig. 4 clearly demonstrate that nerve-ending particles suspended in NaCl–KCl mixture solutions swell markedly, when the external K^+ concentration, $[K]_0$, exceeds a certain limit, $[K]_0^* \approx 30$ mM. Such an increase in the particulate volume in the presence of high K^+ can be expressed as a generalized Boyle–Van 't Hoff's relation, (Eqn 6 or 6'). In the study of Marchbanks[16], the swelling of nerve-ending particles in high K^+ media was not observed. His experiments were made in hypertonic media (0.8 M sucrose), for which the swelling was expected to be very small (Fig. 5A). Our finding is, therefore, not contradictory with his results.

Such a K^+-induced swelling is not only independent on whether or not Na^+ is present in the suspending media (Fig. 4), but is also hardly affected by ouabain, *N*-ethylmaleimide and some metabolic inhibitors such as cyanide and amytal, while it attains a nearly steady level at most within 10 min. It seems highly improbable, therefore, that some active transport mechanism of K^+ participates in the swelling phenomena reported here. It seems to be rather better explained by a passive physical mechanism.

It has been reported that nerve-ending particle membranes were impermeable to SO_4^{2-} but fairly permeable to Cl^- (ref. 17). Our results showed that neither Cl^- nor SO_4^{2-} have any significant effect on the K^+-induced swelling, a finding which excludes the possibility that K^+ is no longer osmotically effective because of simple passive fluxes of K^+ and its counterion.

The swelling which obeys Eqn 6' is also observed with Rb^+ and Cs^+, but never with Na^+ and Li^+. The order of the potency to induce swelling is given by Sequence 7 which resembles quite well that of the externally applied cations for producing the depolarization of the axon membrane.

With our light-scattering method, the effect of raised Ca^{2+} concentrations on K^+-induced swelling could not be successfully studied. But the enhancement of the swelling by some chelating agents (Fig. 9) in parallel with their Ca^{2+}-binding power could be explained by deprivation of membrane-bound Ca^{2+} and might be regarded as an indirect evidence for the action of the Ca^{2+} which counteract the K^+ which induce the swelling.

All these results seemed to suggest that the K^+-induced swelling is closely related to the depolarization of nerve-ending particle membrane. It seems difficult, however, to explain the K^+-induced swelling on the basis of water inflow caused by an electroendosmotic effect due to such a decrease in the membrane potential alone.

At the present stage of investigation, it seems rather reasonable to assume some interaction of the membrane with K^+ which results in structural changes. Indeed, occurrence of swelling in the nerve fibers in an excited state was already noted not only by Bryant and Tobias[18] but also recently by Cohen and Keynes[19] with more refined light-scattering techniques. On the other hand, the changes in light-scattering during synaptic activity were reported by Erinagae *et al.*[20]. The nerve-ending particle swelling induced by high external K^+ concentration reported here may have some correlation with these physiological changes associated with nerve activities.

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