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THE STIMULATION OF Na^+ UPTAKE IN FROG SKIN BY URANYL IONS *

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Summary

1. The Na^+ uptake in the isolated frog skin of *Rana esculenta* was measured by the short-circuit current (I_{sc}). Uranyl ions increase at pH 5.5 the I_{sc} up to 200% at concentrations of 10 mM. The half-maximal value for this effect is at about 1 mM uranyl salt.

2. The effect is (a) specific for the Na^+-transporting pathways in the outer Na^+-selective membrane, (b) fully reversible. No stimulation can be seen in presence of 1 mM H^+ or 0.1 mM amiloride.

3. The decrease of the sodium permeability of the apical membrane (P_{Na}), normally induced by increasing concentrations of Na^+ in the mucosal solution, $[Na]_0$, is partially prevented by uranyl ions. The apparent Michaelis constant of the saturable Na^+ uptake is shifted to much higher values.

4. A comparison between the uranyl effect and similar effects of other drugs leads to the conclusion that uranyl ions might act in a polar hydrophobic environment, possibly by combining with phosphate groups (of phospholipids), and, thus, enhancing Na^+ permeability by changes in tertiary structure near each Na channel. The interaction of mucosal Na^+ with their receptor, normally triggering the $[Na]_0$-dependent decrease of P_{Na}, is thought to be diminished by uranyl association in a neighbouring region, causing a non-competitive stimulation of the Na^+ translocation through the apical frog skin membrane.

Introduction

Many tissues show drastic changes in their ion permeabilities, when exposed to solutions containing ions of a high electrical field strength, e.g. protons or

* A part of the results was briefly reported at the Spring meeting of the Deutsche Physiologische Gesellschaft (1976)

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Abbreviation: PCMB, *p*-chloromercuribenzoate.

polyvalent inorganic cations. The action of protons on Na^+ uptake in frog skin has been reinvestigated by Zeiske and Lindemann [1]. They were able to demonstrate a large inhibitory proton effect at a mucosal pH value of 3, and a slight stimulating effect between pH 6 and 4. The chemical group responsible for the blockage of Na^+ uptake after protonation is thought to be a carboxylic group [1], whereas the nature of a possibly different stimulating group has not yet been determined. The aim of this paper is to show the interaction of uranyl ions with a stimulating group.

It is well known that organic Hg compounds [2—4] and polyvalent inorganic cations like La^{3+} [5], Zn^{2+} [6], Cd^{2+} [6,7], as well as Cu^{2+} [8] and Ag^+ [9] are able to increase Na^+ uptake in frog skin. The mechanism of this process was until now either not discussed or seen as interaction of the ions with sulfhydryl groups [3,4,9] or those Ca^{2+}-binding sites, which are also involved in the action of neurohypophysal hormones [7]. Curran [9] briefly remarks that uranyl ions enhance Na^+ uptake in frog skin, however, without detailed studies or interpretation.

The experiments discussed in this paper were performed in an effort to investigate the influence of uranyl ions at pH 5.5 on the short circuit current (I_{sc}), epithelial resistance (R_M), as well as on the Na^+ permeability of the apical frog skin membrane (P_{Na}). From the experiments it is concluded that the interaction of uranyl ions with appropriate negative sites near the Na channel in the apical membrane increases the P_{Na} of this barrier.

A brief description of a part of the results was presented at the 1976 Spring meeting of the Deutsche Physiologische Gesellschaft [10].

Methods

All experiments were carried out with abdominal skins of *Rana esculenta* by changing the mucosal solutions in an Ussing-type chamber and measuring the resultant changes in I_{sc}, R_M or G_M (conductance), in short circuit conditions. The method of Fuchs et al. [11,12] was used to calculate the P_{Na} of the apical membrane from the Na^+ current, which is assumed to be that part of the I_{sc}, which can be blocked by amiloride [13—15]. The experiments were done in a condition of high serosal K^+ concentration, where the resistance and voltage drop across the laterobasal membrane are relatively small [16]. This was achieved by pre-equilibration of the skins in potassium sulfate-Ringer. Then they were mounted on an agar plug made of potassium sulfate-Ringer, that served as serosal medium.

The change of solutions was performed by injecting a test solution into the chamber compartment containing the outer bathing solution, after a 15 min pre-equilibration period. The solutions were injected in fractions of a second, in a direction parallel to the outer skin surface (1 cm^2) which permitted exchange of solution volume (about 1 ml) in a very short time. The stirring normally stopped after the exchange. For pH titration, the solution flow was held at a slow rate after exchange (5 ml/s) until a steady state was reached.

I_{sc} and R_M (or G_M) values were read from the simultaneously plotted curves at about 25 s after each solution change, i.e. in a steady state. The G_M values

were equal to the ratio $\Delta I/\Delta V$ of a I_{sc} deflection (ΔI) resulting from a short voltage clamp pulse (ΔV) of 5 mV.

All solutions contained 1 mM calcium gluconate, 5 mM Tris, and the sulphates of potassium and/or sodium, the sum of which was kept at 100 mequiv./l. Uranyl salts as well as amiloride (Merck, Sharp and Dohme) were dissolved using magnetic stirring; the pH of the solutions was adjusted with H_2SO_4.

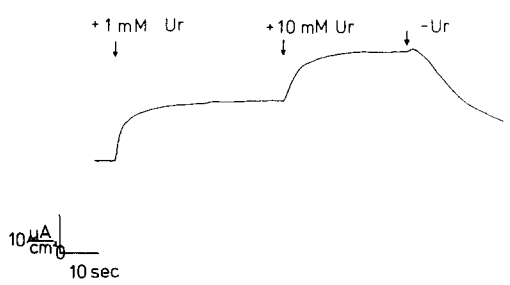
Results

Fig. 1 shows the time course of the I_{sc} after addition of 1 and 10 mM uranyl nitrate in a solution containing 100 mM Na^+ (pH 5.5) to the outer surface of the frog skin. With 10 mM uranyl nitrate the I_{sc} rises within a few seconds, stabilizing at a value which reaches approx. 200% of the original I_{sc} value. After removal of uranyl, a rather rapid decrease of the current indicates a good reversibility of the effect.

The transepithelial resistance was, after addition of 5 mM uranyl, reduced from 620 to 420 $\Omega \cdot cm^2$.

The tendency of uranyl salts to hydrolyze in aqueous solutions and, thereby, to acidify them, makes the use of uranyl rather complicated. Also, high pH values permit the formation of insoluble uranyl or even negatively charged complex uranates [17—20]. For these reasons the effect of the pH on the I_{sc} in uranyl-containing solutions was tested (Fig. 2).

A sudden increase in the Na^+ concentration of the outer solution, $[Na]_0$, from zero to high values normally results in an initial rise of the I_{sc} to a peak value, followed by a "recline" to a plateau which is reached after about 10 s. This phenomenon has been intensively studied by Lindemann and coworkers [12,21,22] using a rapid flow chamber which allows solution changes in 30 ms. The "recline" is due to a decrease of the P_{Na} of the outer membrane brought



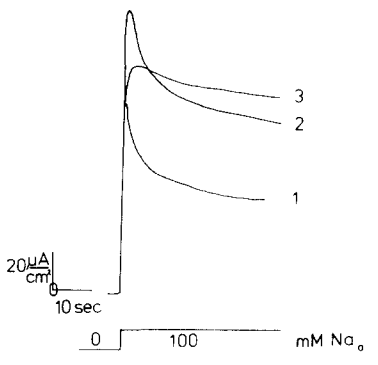


Fig. 1. Increase of short circuit current after the addition of 1 and 10 mM uranyl nitrate (Ur) to the outer solution containing 100 mequiv. sodium sulfate/5 mM Tris/1 mM calcium gluconate at pH 5.5.

Fig. 2. Increase of short circuit current after mucosal substitution of 100 mequiv. K^+ by 100 mequiv. Na^+, curve 1 being the control at pH 7.0, curve 2 with 10 mM uranyl nitrate at pH 7.0, curve 3 with 10 mM uranyl nitrate at pH 5.5.

about within a few seconds by the high [Na] on the outside surface of the skin. (This process was recognized to be responsible for the observed saturation behaviour of Na^+ transport through frog skin [12,21,22]. With the slower flow rates during the K^+-Na^+ exchange in Fig. 2 (curve 1) a curve is obtained with a shape similar to a recline. It must be mentioned, however, that the recline is dominant merely during the first 10 s, whereas the following slow current decrease is due to an increase of the cellular Na^+ concentration, thereby reducing the apical Na^+ concentration gradient and consequently the I_{sc}. Therefore the peak value of the current in Fig. 2 is not the real peak value after a K^+-Na^+ exchange, but somewhat smaller. Repeating the Na^+ concentration jump at pH 5.5 does not increase the plateau level in this experiment. Curve 2 shows the influence of 10 mM uranyl nitrate in the Na^+-containing solution on I_{sc} first at pH 7.0, curve 3 at pH 5.5. The result that uranyl action in a slightly acidic solution was increased, led to the decision not to perform the experiments with uranyl ions at neutral pH. Thus hydrolysis, complex formation, and undefined ionic states of the uranyl cation were avoided. Unless otherwise noted, experiments were therefore carried out at pH 5.5.

To check the influence of uranyl on unspecific paracellular pathways, I_{sc} and R_M were recorded in a Na^+-free solution with 100 mM K^+ at pH 5.5.

Fig. 3 shows that 10 mM uranyl nitrate, when present in the outer bathing solution, decrease I_{sc} and R_M somewhat (a), whereas 10 mM uranyl acetate appear to have no effect on these membrane parameters (b). This difference could be due to differential anion permeabilities, for the frog skin has a significantly higher permeability for nitrate than for acetate [23].

A more convincing proof of the specificity of the uranyl effect on Na^+ uptake can be obtained from the use of amiloride, a specific Na^+ influx blocking agent [13—15]. In the presence of amiloride neither Na^+ current nor the uranyl effect can be seen anymore (Fig. 4).

The dose vs. response curves of the uranyl effect at two different Na^+ concentrations are plotted in Fig. 5A, showing saturation characteristics.

From Fig. 5A it can be seen that an apparent Michaelis constant for the half-maximal uranyl effect, K_m, should have a value of about 1 mM. Fig. 5B shows a double-reciprocal plot of the data of Fig. 5A. It can be seen that K_m, the reciprocal negative value of the abscissa intercept, has a value of 0.8 mM and is not shifted by different $[Na]_0$. Therefore, it must be assumed that the "uranyl-

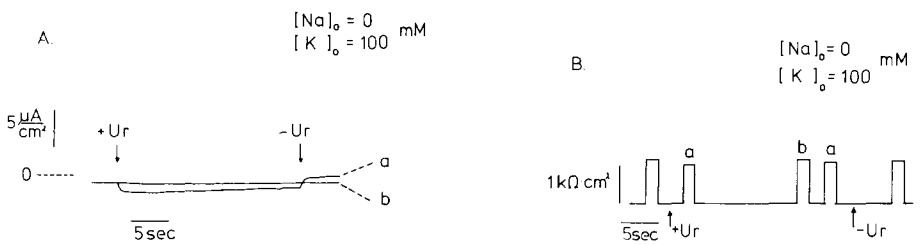


Fig. 3. (A) Effect of 10 mM uranyl nitrate (a) and acetate (b) on I_{sc} in Na^+-free solutions. (B) Effect of 10 mM uranyl nitrate (a) and acetate (b) on epithelial resistance R_M in Na^+-free solutions. (Solutions: 100 mequiv. potassium sulfate/1 mM calcium gluconate/5 mM Tris, pH 7.)

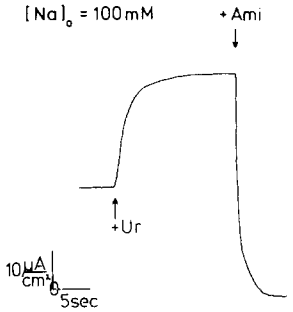


Fig. 4. Increase of I_{sc} by 10 mM uranyl nitrate and blockage of the uranyl effect by 10^{-4} M amiloride (solutions like in Fig. 1).

receptor" is not identical to the "Na-receptor", the occupation of which with Na^+ leads to the decrease of P_{Na} during the recline.

If uranyl ions are able to combine with apparently negative membrane groups, the chemical nature of these groups is of interest. In an attempt to identify these groups, a pH titration curve of the frog skin was obtained by performing K^+-Na^+ substitutions at different pH values of the Na^+-containing solution ($[Na]_0$ = 100 mequiv.). The solutions were injected and then held at a flow rate of about 5 ml/s until a plateau value of the I_{sc} was reached. Fig. 6A shows plateau values of the resultant I_{sc} plotted as function of the outer pH.

In the control curve a relative maximum of Na^+ uptake can be seen which represents the combination of a stimulating first and an inhibitory second pH effect. The second pH effect was previously recognized to be a decrease of P_{Na} after protonation of a carboxylic group near the Na channel [1]. This group

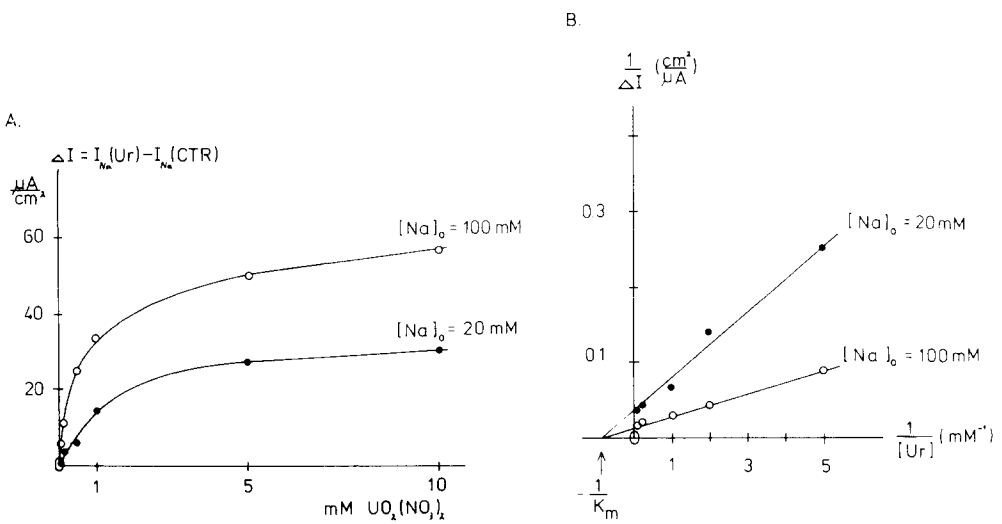


Fig. 5. (A) Dose vs. response curve of the uranyl effect on the I_{sc} for two different Na^+ concentrations (solutions: 20 mequiv. Na^+/80 mequiv. K^+, respectively, 100 mequiv. Na^+/0 mequiv. K^+/50 mM sulphate/1 mM calcium gluconate/5 mM Tris, pH 5.5). (B) Double-reciprocal plot of the I_{sc} increase evoked by uranyl nitrate versus uranyl concentration at two different outer Na^+ concentrations.

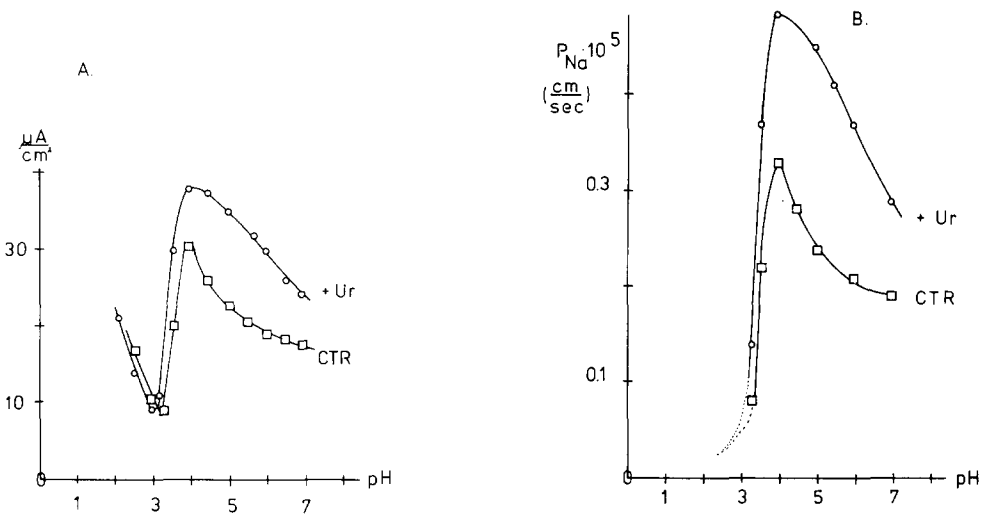


Fig. 6. (A) Short circuit current as a function of the outer pH value without (CTR) and with 1 mM uranyl nitrate at 100 mequiv. $[Na]_0$ (solutions like in Fig. 1, pH adjusted with H_2SO_4). (B) Na^+ permeability (P_{Na}) as function of the outer pH value without (CTR) and with 1 mM uranyl nitrate at 100 mM $[Na]_0$.

seems to be identical to the Na-receptor of the recline inducing center (ref. 24 and Zeiske, W., unpublished). Below pH 3 the I_{sc} increases again: This 3rd pH effect is thought to be mainly a proton influx, since in presence of amiloride, the Na^+ current disappears, and the I_{sc} remains large only at pH <3 [1]. Thus, between pH 3 and alkaline pH values, the Na^+-transporting structures in the outer membrane are specifically titrated.

In the presence of 1 mM uranyl nitrate, currents above pH 4 were larger. The Na^+ permeability of the apical membrane, P_{Na}, calculated according to Fuchs et al. [11,12], reflects this increase in current (Fig. 6B). The increase is even more pronounced for P_{Na} than for the short-circuit current. Below pH 4, however, the stimulating uranyl effect seems to disappear. This cannot be due simply to a replacement of uranyl at its binding sites by protons, since the interference of uranyl ions with the 1st pH effect (smoothing of the curve at pH >4) indicates rather a similar P_{Na}-increasing action of UO_2^{2+} and H^+ than a direct inhibition of the uranyl effect by protons. Therefore, the decrease of P_{Na}, which can be seen below pH 4 for both curves in Fig. 6B, is thought to be a result from a reduction of P_{Na} through the 2nd pH effect, thereby making any stimulating action more or less undetectable. To investigate the interference of uranyl with the 1st pH effect further, a special kinetic analysis of P_{Na} was made.

Lindemann and co-workers [11,12,21] have shown that the saturation behaviour of frog skin is due to a decrease of P_{Na} with an increase of the mucosal Na^+ concentration. This process is rather rapid so that its time course could only be detected in experiments with a rapid flow chamber (the slower solution-injection method used for the experiments in Fig. 2 does not preserve the time course of the P_{Na} decrease to the same extent. It does, however, permit to observe the steady-state response already 20 s after the change of solution). In the framework of the kinetic model of steady-state P_{Na}, an equation was formulated for the reaction of mucosal Na^+ at a certain concentra-

tion $[Na]_0$ with a receptor site (apparent dissociation constant K_{Na}), which describes the decrease of P_{Na} with increasing $[Na]_0$ from a maximal value P^0_{Na} at zero $[Na]_0$ [11,12,21]:

$$P_{Na} = P^0_{Na} \cdot \frac{K_{Na}}{K_{Na} + [Na]_0} \quad \text{(I)}$$

Rearrangement of Eqn. I yields a linear function $1/P_{Na} = f([Na]_0)$:

$$\frac{1}{P_{Na}} = \frac{1}{P^0_{Na}} + \frac{1}{K_{Na} \cdot P^0_{Na}} \cdot [Na]_0 \quad \text{(II)}$$

Fig. 7 shows such a plot without (CTR) and in presence of 1 mM uranyl ions. A competitive blocker like amiloride shifts the line upwards in a parallel manner (dashed line, according to refs. 22 and 24). Uranyl ions, however, induce a clockwise rotation around the ordinate intercept. This is already known from the action of the mercury compound PCMB [3,22] and some guanidine derivatives of benzimidazole (ref. 22 and Zeiske, W., unpublished) and benzothiazole (ref. 12 and Zeiske, W., unpublished). Low proton concentrations show the same behaviour (1st pH effect, ref. 22 and Zeiske, W., unpublished). At very low Na^+ concentrations, a deviation of the experimental data from the predicted straight line can be observed. The value of $1/P_{Na}$ at comparable $(UO_2^{2+})/(Na^+)$ ratios is even smaller than $1/P^0_{Na}$. This could perhaps be due to a second stimulating action of uranyl ions on Na^+ transport, probably at a site different from the "normal" uranyl receptor. For higher Na^+ concentrations, Eqn. II obviously describes the experiments sufficiently.

With respect to Eqn. II, a decrease of the slope and a constant ordinate intercept mean that the apparent K_{Na} has to increase under the influence of uranyl ions and similarly acting substances: Now higher Na^+ concentrations are

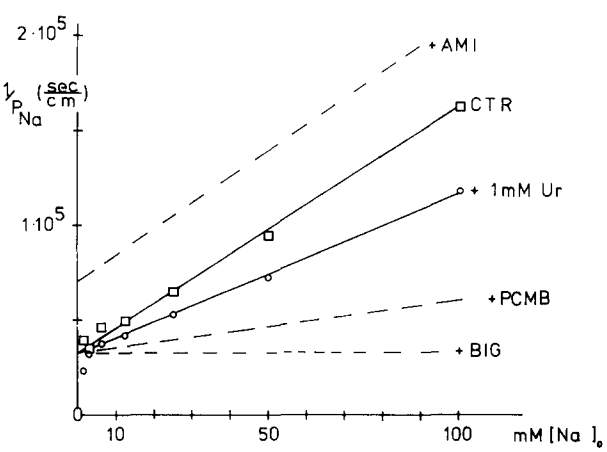


Fig. 7. Reciprocal Na^+ permeability (P_{Na}) plotted versus $[Na]_0$ without (CTR) and with 1 mM uranyl nitrate. CTR curve is shifted upwards by amiloride (AMI) and rotates clockwise in presence of uranyl ions (Ur), *p*-chloromercuribenzoate (PCMB) and benzimidazolyl-2-guanidinium (BIG). (Solutions with 1 mM calcium gluconate/5 mM Tris (pH 5.5)/50 mM sulfate, and the sum of K^+ and Na^+ concentration always being 100 mequiv.) The dashed lines for amiloride, PCMB and benzimidazolyl-2-guanidinium approximately represent earlier findings (ref. 3, 22 and Zeiske, W., unpublished).

needed to induce the $[Na]_0$-dependent decrease of P_{Na}. Thus, K_{Na} becomes very high already at low concentrations of PCMB or benzimidazolyl-2-guanidinium. It could be shown that the latter P_{Na}-increasing substances make P_{Na} independent of $[Na]_0$ at sufficiently high concentrations (refs. 3, 25 and Zeiske, W., unpublished).

Discussion

The experiments described herein show that the stimulating uranyl effect is (a) Na^+ specific and (b) reversible. The inhibitory effect of protons at pH 3 and of the specific Na^+-blocking drug amiloride both override the uranyl effect. Non-specific cellular or paracellular shunt pathways seem to be unaffected by uranyl.

To what part of frog skin epithelium do uranyl ions bind?

The reasonably fast injection technique used and the relatively fast response of the skins to the addition of uranyl indicates that the reaction with the appropriate sites is not intracellular, but rather on the outer surface of the Na^+-selective membrane of the outermost living cell layer. The probability for uranyl ions to reach the cell interior is small not only (a) because of their tendency to react with many membrane components, but also (b) because of their probably low membrane permeability. The permeability to uranyl ions of the apical membrane should be as small as that to lanthanum ions, which also increase the Na^+ current [5].

A number of reports from this laboratory have dealt with the effect of drugs on the $[Na]_0$ dependence of P_{Na}. The action of amiloride [22,24,25] protons at pH 3 (ref. 1 and Zeiske, W., unpublished) and 2,4,6-triaminopyrimidine [24] on the receptor for Na^+ which is responsible for the Na^+-induced P_{Na} reduction could be interpreted as synergistic. On the other hand, protons at pH 5 (ref. 22 and Zeiske, W., unpublished), drugs like benzimidazolyl-2-guanidinium (ref. 22 and Zeiske, W., unpublished) and PCMB [3,22] diminish, to a certain degree, the P_{Na} reduction by mucosal sodium.

Uranyl ions seem to have the same effect as benzimidazolyl-2-guanidinium and PCMB, namely, to reduce the potency of Na^+ to decrease their own permeability after binding to a certain site on the apical membrane. This site is assumed to be located close to the Na^+-transporting macro-molecule. Recent investigation suggested these structures to have the character of channels through which Na^+ transfer occurs by simple electrodiffusion [12,26]. In agreement, it was shown that this transport is passive [27]. Transport saturation through Na channels has been found to be not a result of a saturation of the Na channel itself. Rather the P_{Na} reduction of the apical membrane appears to result from the closure of an increasing number of channels with increasing concentrations of Na^+ in the mucosal solution [28].

Does uranyl act in the UO_2^{2+} form?

The soluble uranyl salts like the chloride, nitrate or acetate are able to change their ionic state at different pH values. Highly acidic solutions contain almost exclusively pure UO_2^{2+}, neutral or basic solutions readily form insoluble

polycations, $UO_2(OH)_2$, hydroxy salts or even soluble anionic uranato complexes [17—20]. Therefore, it was generally advantageous to perform the experiments in solutions of pH <7. A criterium for the presence of UO_2^{2+} was a clear yellowish colour of the solutions which might have contained additionally a basic cation like UO_2OH^+ or even cationic uranyl polymers at pH >6—7 [20,29]. In any case, it is certain that the reacting ion could never be an anionic unranate complex, which is formed only at rather high pH values.

At the same time, anionic complexes of uranyl with anions like sulfate as ligands can be excluded at a pH below 6 [20]. Therefore, it is likely that a positive form, probably the positive ion UO_2^{2+}, is responsible for the uranyl effect. Additional support for this suggestion is the recent finding that the hydrated ions of calcium, zinc and cadmium are able to evoke an analogous P_{Na} stimulation in frog skin (Zeiske, W., unpublished).

To which chemical structures does uranyl bind?

While Zn^{2+} and Cd^{2+} react with mercapto groups, La^{3+} and Ca^{2+} cannot do this. UO_2^{2+} too does not react with SH [30]. Nevertheless, all five ionic species increase P_{Na}. It is clear, therefore, that the common denominator of the consistent stimulating effects of polyvalent cations on Na^+ permeability cannot be binding of these ions to mercapto groups. The apparent pK value for the group titrated between pH 4 and 7 lends additional support to this conclusion. More likely binding sites serving as ligands for a complex with UO_2^{2+} are negative groups like carboxylate and phosphate or dipolic structures like free or substituted amino groups. However, the latter can be excluded to be the uranyl receptor due to the fact that these groups are protonated below pH 7 and, thus, are unable to bind positive ions like UO_2^{2+}. Therefore carboxylate and phosphate are the likely candidates for the uranyl receptor at the membrane (compare ref. 31). Their influence on P_{Na} has been previously discussed [1]. It has been shown that combination of carboxylate with cations of high field strength, e.g. H^+, results in a decrease of P_{Na}. This "second pH effect" resembles the effect of Na^+, amiloride or 2,4,6-triaminopyrimidine on P_{Na}, but is just opposite to the uranyl effect. Uranyl stimulates by inhibition of the $[Na]_0$-dependent P_{Na} reduction. The same mechanism has been shown to be responsible for the action of benzimidazolyl-2-guanidinium (ref. 22 and Zeiske, W., unpublished), PCMB [3,22] and low proton concentrations ("first pH effect" (ref. 22 and Zeiske, W., unpublished). They all cause a clockwise rotation of the straight control line in the $1/P_{Na} - [Na]_0$ plot (cf. this paper Fig. 7 and ref. 22, Fig. 16). Especially the similarity of the 1st pH effect to the action of uranyl ions is of interest, because the protonation of a membrane component between pH 4 and 7 must interfere with the likewise stimulating action of uranyl ions (cf. Fig. 6B). The turning point of the control curve in Fig. 6B between pH 4 and 7 seems to lie in a more acidic range and may be masked by the onset of the 2nd pH effect. Zeiske (unpublished) found an apparent pK for the group titrated between pH 4 and 7 of 4.3, which is similar to that of carboxylic and phosphoric acids in free solution.

But an interaction of protons only with carboxylic groups which are the sites for the 2nd pH effect, would rather result in a decrease of P_{Na} and thus in an inhibition than in stimulation. However, a comparison of the measured pK

values of titrated membrane components with those known from titrations in free solution may easily lead to wrong conclusions, since dissociation constants in a rather hydrophobic region near a membrane might be changed severely. It is therefore useful to look for some additional arguments concerning the site of uranyl action. Zeiske (unpublished) could also show a stimulation of Na^+ uptake by chemically rather different anorganic ions such as Ca^{2+} and Zn^{2+}, which was caused by exactly the same mechanism like the uranyl or the 1st pH effect. Since it is well known that the phosphates of UO_2^{2+}, Ca^{2+} and Zn^{2+} are all very stable, it is assumed that phosphate groups are the likely site for the stimulating action of these anorganic cations, including H^+ in the 1st pH effect. High H^+ concentrations or high doses of amiloride can still overcome the action of the stimulators, probably by closing the Na channel after interaction with a carboxylic group in the neighbourhood (refs. 1, 24 and Zeiske, W., unpublished) and thus eliminating Na^+ transport which is the only indicator for any stimulating effect.

The strong binding to inorganic or organic phosphate groups, possibly in the neighbourhood of hydrophobic regions in the membrane, is a common characteristic of many heavy metal ions, e.g. La^{3+}, Zn^{2+}, Cu^{2+}, Hg^{2+}, Ag^+ or Cd^{2+}. They all are able to combine strongly with polarizable negative groups like phosphate [31] and stimulate Na^+ influx [5,6,8,3,9,7]. Further substantiation for this interpretation can be found in the use of uranyl salts in electron microscopy techniques; they are throught to bind to phosphate groups of phospholipids [33].

On the other hand, benzimidazolyl-2-guanidinium may share its mechanism of action to a certain extent with UO_2^{2+}. While similarities in the chemical structure of uranyl ions and benzimidazolyl-2-guanidinium are not apparent, two common structural features can nevertheless be discussed: (a) both agents carry net positive charges [25], and (b) both are soluble in an organic environment of a decreased hydrophilic nature [20,25,33].

In this context, other Na^+ uptake enhancing drugs like harmaline [34], novobiocine [35], diphenylhydantoine [36] and barbiturates (Zeiske, W., unpublished) should be mentioned. The mechanism of their action has not yet been explained, but their rather hydrophobic molecular structures might be involved in the association to a membrane region with a certain lipophilic character. The actions of diphenylhydantoine and barbiturates on nerve membranes are attributed to such a behaviour [37].

It is true that the action of PCMB must not, as usually thought, involve only SH groups [22], since PCMB may also combine for example with carboxylate or phosphate. However, organic mercurials are probably the most specific SH-reagents known [32]. Therefore, it seems at least likely that the PCMB-receptor is an SH-group. In this case the receptor for PCMB and uranyl ions would be different.

The interpretation of the uranyl effect

All these facts support the conclusion that between pH 5 and 7 uranyl ions appear to combine with negatively charged phosphate groups at the phase boundary "lipid-protein" in the apical membrane. A likely ligand for the uranyl ions is the phosphatidylcholine molecule. The solubility of uranyl salts in polar

hydrophobic regions favours this association.

The association of phosphate and UO_2^{2+}, which is known to be rather strong [31], could give rise to a change in tertiary structure of complex lipid-protein molecules near the site which is responsible for the P_{Na} reduction by Na^+, amiloride or protons, i.e. near the "Na channel" in the Na^+-selective apical frog skin membrane. The P_{Na} decrease normally mediated by Na^+ is, thus, blocked to a certain degree by UO_2^{2+} (The same mechanism may be assumed for the stimulation of Na^+ uptake by small inorganic cations, including protons in the 1st pH effect). In the presence of highly concentrated protons or amiloride, which still are more potent P_{Na} reductors than Na^+ themselves (ref. 1, 22, 24 and Zeiske, W., unpublished), uranyl ions can no more overcome the effect of these strong blockers of Na^+ uptake.

Direct screening of the Na-receptor was tentatively considered to be the mechanism of action of large P_{Na}-stimulating molecules like benzimidazolyl-2-guanidinium and PCMB [22]. In contrast, the small uranyl ions, which bind strongly to phosphate moieties, will hardly screen directly. However, after binding to phosphate they may involve neighbouring polarizable groups by complex formation and thereby change the geometry of the Na-receptor. Association of Na^+ at its receptor site would thus be inhibited and the $[Na]_0$-dependent reduction of P_{Na} suppressed. In this way agents of rather different structures and different binding sites would exert the same observable effect.

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