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Effect of the Putative Ca^{2+}-Receptor Agonist Gd^{3+} on the Active Transepithelial Na^+ Transport in Frog Skin

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Abstract. In this communication we show that Gd^{3+} acts as an activator of the apical sodium channel (ENaC) in frog skin epithelia.

Application of Gd^{3+} to the apical solution of frog skin epithelia increased the Na^+ absorption measured as the amiloride-inhibitable short-circuit current (I_{sc}). The stimulation was dose dependent with a concentration for half-maximal stimulation (EC_{50}) of 0.023 mM. The change in I_{sc} was found to correlate with the net Na^+ flux, confirming that Gd^{3+} enhances Na^+ absorption. By monitoring the cellular potential (V_{sc}) with microelectrodes during addition of Gd^{3+}, it was found that V_{sc} depolarized as I_{sc} rose, indicating that Gd^{3+} affects apical Na^+ permeability (P_{Na}). This was confirmed by measuring the I/V relations of the apical membrane.

In the presence of benzimidazolylguanidin (BIG), a drug known to abolish the Na^+ self-inhibition, Gd^{3+} had no effect on I_{sc}. The Na^+ self-inhibition was investigated using fast changes of the apical Na^+ concentration on K^+-depolarized epithelia. BIG was found to abolish the Na^+ self-inhibition and to activate the basal Na^+ transport, whereas Gd^{3+} only activated the basal Na^+ transport but had no effect on the self-inhibition. These results indicate the existence of an alternative nonhormonal mechanism to Na^+ self-inhibition, via which both Gd^{3+} and BIG act, possibly components of the Na^+ feedback inhibition system.

Key words: Frog skin — Sodium transport — Gadolinium — Self-inhibition — Feedback inhibition

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Introduction

By the use of the isolated frog skin as experimental object and radioactive isotopes as tools for measuring ion transport, Hans H. Ussing developed, based on his profound scientific intuition, the conceptual means by which the transport (active and passive) of ions across membranes can be identified and interpreted. Until today investigators working on transport of ions and metabolites use the flux-ratio equation to distinguish between active and passive transport (Ussing, 1949; Sten-Knudsen & Ussing, 1981), the short-circuit technique to measure active transport (Ussing & Zerahn, 1951) and the two-membrane hypothesis as the basic framework to interpret the observation (Koefoed-Johnsen & Ussing, 1958).

The abdominal skin of frog contains an absorptive as well as a secretory system for NaCl and water. The absorptive system is located in the epithelium and the secretory system resides in the exocrine skin glands. The active transepithelial Na^+ absorption is carried out by the principal cells, whereas Cl^- follows passively either via the mitochondria-rich cells or by the paracellular pathway (Larsen, Ussing & Spring, 1987; Nagel, Garcia-Diaz & Essig, 1983).

Ussing and coworkers investigated the single-membrane properties of the frog skin by the use of Ringer's solutions containing impermeable anions (sulphate) and different concentrations of K^+ and Na^+. They found that the apical and basolateral membrane behaved as a Na^+- and a K^+ electrode, respectively. This led to the formulation of the two-membrane hypothesis, which describes the active uptake of Na^+ via the epithelium (Fig. 1). According to this, Na^+ transport takes place when apical Na^+ diffuses down an electrochemical gradient across the apical membrane and is extruded via the Na^+/K^+-pump located in the basolateral membrane in

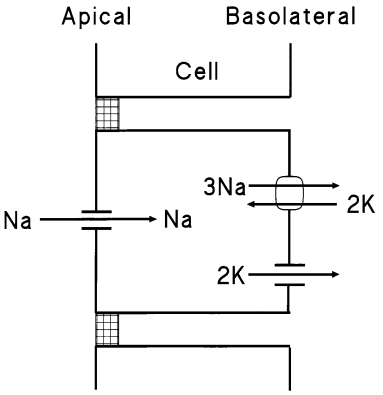

Fig. 1. The two-membrane hypothesis.

exchange for K^+ that recycles via K^+ channels in the basolateral membrane (Koefoed-Johnsen & Ussing, 1958). The coupling ratio of the Na^+/K^+-pump is 3:2 (Nielsen,1979).

Na^+ passes the apical membrane via channels that have electrophysiological characteristics identical to the amiloride-sensitive epithelial Na^+ channels (ENaC), i.e., high Na^+:K^+-selectivity, high affinity for amiloride and a relatively low conductance. The channel complex consists of 2 α-,1 β- and 1 γ-subunit, the α-subunit containing the amiloride binding site (Ismailov et al., 1997).

Regulation of the number of Na^+ channels in the membrane and their activity has a great influence on the active Na^+ transport.

The Na^+ uptake across the apical membrane into the organism is under hormonal control, working on a relatively slow timescale. The active uptake of Na^+ across the isolated frog skin is activated by antidiuretic hormone (ADH) (Koefoed-Johnsen & Ussing, 1953), β-adrenergic agonists such as noradrenalin (Gudme, Larsen & Nielsen, 2001) and prostaglandin E_2(PGE_2) (Rytved, Brodin & Nielsen, 1995). These effects are mediated via V_2-, β-adrenergic and EP_2-receptors, respectively, all leading to an increase in the cellular production of cAMP (Johnsen & Nielsen, 1978; Rytved et al., 1995). Another way to increase the Na^+ absorption is by activation of P_2Y-receptors. This effect is mediated by an increase in $[Ca^{2+}]_i$ (Brodin & Nielsen, 2000). This is in contrast to the effect of an increase in $[Ca^{2+}]_i$ mediated by inhibition of the endoplasmatic Ca^{2+} pumps, which inhibits the transepithelial Na^+ transport (Brodin, Rytved & Nielsen, 1996). Furthermore, the Na^+ transport is inhibited by a high concentration of PGE_2 and noradrenaline mediated via EP_3- and alpha-2-adrenergic-receptors due to a reduction in the cAMP production (Rytved & Nielsen, 1999; Gudme et al., 2001).

Measurements of the intracellular potential have shown that PGE_2 and ADH induce stimulation of the sodium transport due to an increase in the Na^+ permeability (P_{Na}) of the apical membrane (Els & Helman, 1981). Noise analysis has shown that ADH and PGE_2 raise P_{Na} by increasing the total number of conducting Na^+ channels (Helman, Cox & Van Driessche, 1983). PGE_2 production can be initiated by addition of agents, which are supposed to increase the cellular Ca^{2+} concentration (Rytved et al., 1995).

To prevent the Na^+-transporting principal cells from Na^+ flooding, additional mechanisms of regulation have been identified, namely, Na^+ self-inhibition and Na^+-feedback regulation (Garty & Palmer, 1997).

The concept of Na^+ self-inhibition is that extracellular Na^+ per se modifies the activity of the Na^+ channel (Lindemann, 1984), whereas feedback regulation of the Na^+ channel is a secondary effect of changes in intracellular Na^+ concentration brought about by changes in the intracellular concentration of other ions and various metabolites, e.g., pH and Ca^{2+} (Turnheim, 1991).

Materials and Methods

The experiments were performed on skin from male and female frogs (*Rana esculenta*). The animals were kept at room temperature with free access to water and food.

Solutions

A modified Cl^--Ringer's solution of the following composition (mM) was used throughout the experiments, except in the fast-flow experiments: Na^+ 115; K^+ 2.5; Ca^{2+} 1; Cl^- 119.5; glucose 5; HEPES 5; pH = 7,4. In the fast-flow experiments, modified SO_4^{2-} Ringer's solutions of the following composition were used: (1) K^+ 117.5; Ca^{2+} 1; SO_4^{2-} 58.75; glucose 5; HEPES 5; pH = 7,4 and (2) Na^+ 115; K^+ 2.5; SO_4^{2-} 58.75; glucose 5; HEPES 5; pH = 7,4

Isolation of the Epithelium

The epithelia were isolated by exposing the serosal side of the skin to Cl^- Ringer's solution containing 1.2 mg ml^{-1} of crude collagenase for 75 minutes. After removal of the collagenase-containing solution, fresh Cl^- Ringer's solution was added and a hydrostatic pressure of 20–30 cm was applied to the serosal side. The epithelium was dissected from the dermis when visible blisters had formed.

Ussing-Chamber Experiments

Symmetrical skin halves were mounted in Ussing-chambers (area 1.5 cm^2) and bathed in stirred Ringer's solutions under open-circuit conditions for approximately 1 hour. The experiments were started by replacing the solution on both sides and short-circuiting the epithelium.

Active transport of Na^+ across the epithelium was measured as the amiloride-sensitive I_{sc}, measured according to the technique of Ussing and Zehran, using an automatic voltage clamp, compensating for the potential drop between the potential-measuring electrodes (Ussing & Zerahn, 1951).

Flux Measurements

In these experiments, one of the symmetrical skin halves was used for measuring the influx and the other for the efflux. $^{22}Na^+$ was added to the relevant solution bathing one side of the skin and allowed to equilibrate for 30 minutes. A sample was taken every 30 minutes from the opposite side of addition and the activities of the samples were measured by liquid scintillation. I_{sc} was measured throughout the experiment and the mean values for each 30-minute period were calculated.

Intracellular Measurements

The cellular potential (V_{sc}) was measured with a microelectrode connected to a high-impedance electrode amplifier with the tissue held under short-circuit conditions. Microelectrodes were pulled from 1.2 mm glass capillaries and were backfilled with 0.3 M KCl. The electrodes had a tip resistance of 50–200 MΩ when immersed in the bath solution. The epithelium was mounted in a modified Ussing-chamber with the basolateral side facing upwards allowing perpendicular impalements of the skin with the aid of a motor-driven micromanipulator.

The I/V-relations of the apical membrane were obtained by clamping the transepithelial potential at a series of non-zero values from −140 to +140 mV in steps of 20 mV. The current across the skin (I_{total}) and the potential across the apical membrane (V_{apical}) were measured.

The net Na^+ current across the apical membrane (I_{Na}) was obtained as $I_{Na} = I_{total} - I_{amiloride}$. $I_{amiloride}$ was measured as the current in the presence of 0.1 mM amiloride at the different clamp values.

To estimate the Na^+ permeability of the apical membrane (P_{Na}), the I_{Na}/V_{apical} relation was fitted to the constant-field equation:

$$I_{Na} = -[P_{Na}\beta F][(c^a_{Na} - c^{c}_{Na}{}^{\beta})/(1 - e^{\beta})], \quad (1)$$

where $\beta = FV_{apical}/(RT)$. F,R and T have their usual meaning, c^a_{Na} and c^c_{Na} are the Na^+ concentrations of the apical solution and the cytosol, respectively.

Fast-Flow Experiments

These experiments were performed as described by Fuchs, Larsen & Lindemann, (1977), using a chamber where 1 cm^2 of the apical surface of the skin formed the bottom of a 2-mm high fast-flow channel. The apical surface was constantly perfused with solution at low flow rate (10 ml min^{-1}). The speed of the apical solution was accelerated to 1400 ml min^{-1} just before a change in the apical solution was performed. The changes in solution were carried out by means of an electrically controlled valve so that the apical solution could be changed from Na^+-free to 115 mM Na^+ for 5–6 seconds and back to Na^+-free Ringer's solution again.

Results

The experiments were carried out on isolated epithelia. Addition of 0.3 mM Gd^{3+} to the basolateral solution had no effect or induced a slight inhibition of the short circuit current (I_{sc}). Addition of 0.3 mM Gd^{3+} to the apical solution resulted in an increase in I_{sc} from a control value of 14.8 ± 2.8 to 31.1 ± 4.8 μA cm^{-2} (n = 7). Amiloride was able to block this stimulation, indicating that the observed increase in I_{sc} was caused by an increase in Na^+ transport (Fig. 2).

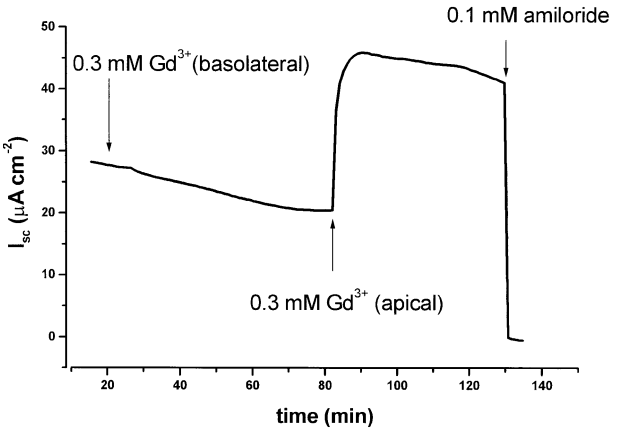

Fig. 2. The effect on I_{sc} of basolateral and apical application of 0.3 mM Gd^{3+}.

The stimulatory effect of Gd^{3+} on I_{sc} was concentration dependent, with an EC_{50} of 0.023 mM (*data not shown*).

The Effect of Gd^{3+} on $^{22}Na^+$ Fluxes

The ionic nature of the I_{sc} stimulation was elucidated by measuring the influx and efflux of $^{22}Na^+$ across symmetrical skin halves. 0.1 mM Gd^{3+} stimulated the influx but had no effect on the efflux, thereby enhancing the Na^+ net flux, which correlates well with the changes seen in I_{sc}. The change from control steady-state to Gd^{3+}-activated steady-state is approximately 10 neq cm^{-2} min^{-1}, measured both as Na^+ net flux and I_{sc}, confirming that it is indeed the Na^+ absorption that is stimulated by Gd^{3+} (Table 1).

Effect of Gd^{3+} on V_{SC}

According to the two-membrane hypothesis, Na^+ is driven across the apical membrane by its electrochemical gradient and is extruded basolaterally by the Na^+/K^+-pump. Augmenting the electrochemical gradient across or the Na^+ permeability (P_{Na}) of the apical membrane will therefore result in an increase of Na^+ transport.

The cellular potential under short-circuit conditions can, consistent with the hypothesis, as first approximation, be described by the Goldmann-Hodgkin-Katz equation:

$$V_{sc} = \frac{RT}{F} \ln \frac{P_{Na}c^c_{Na} + P_K c^c_K + P_{Cl}c^o_{Cl}}{P_{Na}c^o_{Na} + P_K c^o_K + P_{Cl}c^c_{Cl}} \quad (2)$$

where R, T, and F have their usual meaning P_{Na}, P_K and P_{Cl} are the permeability of Na^+, K^+ and Cl^-

Table 1. Effect of Gd^{3+} (0.1 mM apical) on transepithelial Na^+ transport and I_{sc}

Sample (min)	Gd^{3+} (mM)	Influx (neq cm^{-2} min^{-1})	Efflux (neq cm^{-2} min^{-1})	Net flux (neq cm^{-2} min^{-1})	I_{sc} (neq cm^{-2} min^{-1})
0–30	0	9.35 ± 1.35	0.62 ± 0.33	8.72 ± 1.35	9.31 ± 1.51
30–60	0	9.18 ± 1.46	0.86 ± 0.61	8.32 ± 1.67	8.68 ± 1.50
60–90	0.1	12.00 ± 1.22	0.56 ± 0.17	11.44 ± 1.20	13.99 ± 1.87
90–120	0.1	17.87 ± 1.93	0.42 ± 0.11	17.45 ± 1.81	18.11 ± 1.98
120–150	0.1	19.94 ± 1.96	0.32 ± 0.04	19.61 ± 1.90	16.68 ± 1.36

Table 2. Effect of Gd^{3+} (0.1 mM apical) on I_{sc} and V_{sc}

Parameter	Basal (control)	Gd^{3+} 0.1 mM	Amiloride 0.03 mM
I_{sc} (μAcm^{-2})	8.05 ± 0.8	12.4 ± 0.8	1.46 ± 0.4
V_{sc} (mV)	−73.1 ± 3.2	−62.7 ± 4.3	−83.5 ± 4.0

respectively and c_X^c , c_X^o are the concentration of the ion in the cell and in the extracellular bath, respectively.

To investigate the effect of Gd^{3+} on V_{sc}, the epithelium was impaled with microelectrodes from the basolateral side. It was found that concomitant to an about 50% increase of I_{sc}, V_{sc} depolarized 10 mV when 0.1 mM Gd^{3+} was added to the apical bath (Table 2).

Equation 2 predicts that an increase in P_{Na} will cause V_{sc} to depolarize. Closing of K^+- or opening Cl^- channels could also cause a depolarization, but Na^+ absorption would decrease due to the lower electrochemical gradient. This observation, therefore, indicates that Gd^{3+} increases Na^+ absorption by enhancing P_{Na} of the apical membrane.

The Effect of Gd^{3+} on P_{Na}

The change in P_{Na} was investigated by direct measurements of the I_{Na}/V_{apical}-relationship of the apical membrane. I_{Na} plotted as a function of V_{apical} demonstrates Goldmann-rectification and P_{Na} can therefore be found by fitting the curves to the constant field equation (Eq.1).

The permeability was shown to increase significantly from $2.1 \times 10^{-7} \pm 0.6$ cm sec^{-1} to $4.5 \times 10^{-7} \pm 0.4$ cm sec^{-1} after application of 0.1 mM Gd^{3+} ($p < 0.05$; $n = 4$) confirming that Gd^{3+} increases the transepithelial Na^+ transport by increasing P_{Na} of the apical membrane (Fig. 3).

Effect of Gd^{3+} in the Presence of Big

The results obtained so far indicate that Gd^{3+} might act on the Na^+ channel by either releasing it from Na^+ self-inhibition or by interfering with the Na^+ feedback inhibition. In order to elucidate these possibilities the effect of Gd^{3+} in the presence of BIG

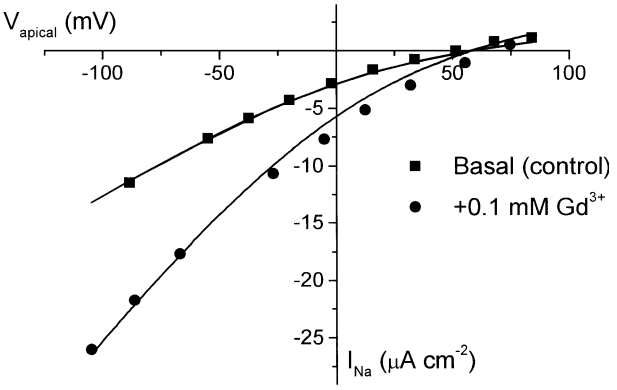

Fig. 3. I/V-relations of the apical membrane before and after addition of 0.1 mM Gd^{3+}. The curves where fitted to the constant field equation yielding an estimate of P_{Na}.

was investigated. BIG is a drug that activates Na^+ transport by releasing apical Na^+ channels from Na^+ self-inhibition.

On the basis of dose-concentration relations, a dose of 1 mM BIG was used since it caused maximal stimulation.

When the tissue was stimulated with this dose of BIG the response of a following maximal dose of Gd^{3+} (0.3 mM) was almost completely abolished, indicating that the two drugs act on the same site. On the other hand, when the tissue was first stimulated with Gd^{3+}, a large response comprising approximately 50% of the total response is seen after the subsequent addition of 1 mM BIG. This indicates that BIG has two different sites of action, sharing one site of action with Gd^{3+} (Fig 4).

Gd^{3+} Does not Act on Na^+ Self-Inhibition

The experiments described above are carried out under steady-state conditions, where it is not possible to distinguish between Na^+ self-inhibition and Na^+ feedback inhibition. In order to differentiate between the two mechanisms, non-steady state measurement of I_{sc} was carried out using the fast flow technique, where I_{sc} was measured during rapid changes of Na^+ concentration in the apical solution. These experiments were performed on epithelia with the electrical properties of the apical membrane. This was obtained by having a K^+ concentration in the basolateral so-

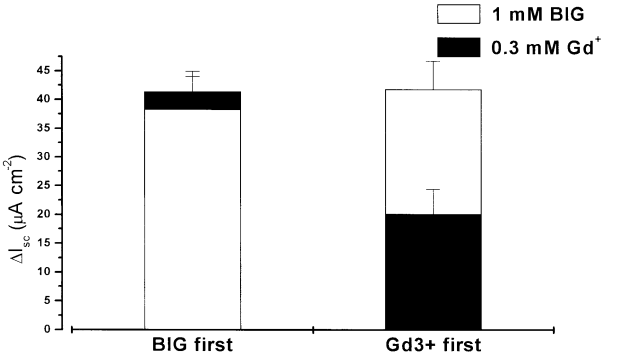

Fig. 4. First column: Effect on I_{sc} of BIG (a dose giving maximum response) followed by a dose of Gd^{3+} (giving maximum response). Second column: Effect of the two drugs added in the reverse order. The bars show the contribution of each drug to the total stimulation.

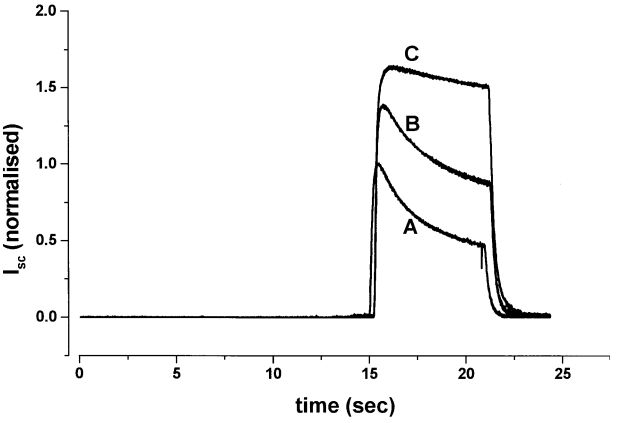

Fig. 5. Three superimposed I_{sc} traces where fast changes in I_{sc} were measured on K^+-depolarized tissue Curve *A*: Under control conditions I_{sc} rises sharply to a peak followed by a recline to a steady-state value due to Na^+ self-inhibition. Curve *B*: Incubation with Gd^{3+} (0.3 mM apical) causes the peak value to rise but the recline is still present. Curve *C*: Addition of both Gd^{3+} (0.3 mM apical) and BIG (1 mM apical) removes the recline. I_{sc} is normalized by setting I_{sc} peak under control conditions equal to 1. Typical experiment ($n = 5$).

lution that approximates the K^+ concentration in the cells. Thus the basolateral membrane was bathed in Na^+-free K_2SO_4-solution. The impermeant SO_4^{2-}-ion was used as the anion in order to avoid excessive swelling of the cells. This causes the basolateral barrier to depolarize and reduces the resistance of the membrane to very low value (Fuchs et al., 1977).

The experiments were carried out under short-circuited conditions. Under symmetrical conditions (in the presence of apical K_2SO_4-Ringer) the I_{sc} was zero. When Na^+ was introduced into the apical solution, there was a sharp increase in I_{sc} (caused by Na^+ influx across the apical membrane) to a peak value followed by a slower recline to a steady-state value. The recline seen is supposedly due to extracellular Na^+ acting via Na^+ self-inhibition (Fuchs et al., 1977).

Epithelia preincubated with 0.3 mM Gd^{3+} had a higher peak I_{sc} but the recline was still present indicating that Gd^{3+} acts via an alternative mechanism, possibly by interfering with one of the mechanisms involved in the Na^+ feedback inhibition system. Incubating the tissue in the presence of 1 mM BIG and 0.3 mM Gd^{3+} resulted in an even higher peak and abolished the recline, showing that BIG is capable of releasing the channels from Na^+ self-inhibition (Fig. 5) as shown before (Zeiske & Lindemann, 1974; Li & Lindemann, 1983). These results thus show that BIG is able to abolish Na^+ self-inhibition but they also reveal an additional mechanism by which both Gd^{3+} and BIG can stimulate the transport of Na^+, by increasing the Na^+ permeability of the apical membrane.

Discussion

From the data presented it appears that addition of Gd^{3+} to the apical surface of the isolated frog skin epithelium results in an increase in the active transepithelial Na^+ transport. This increase is at least partially caused by an increase in the Na^+ permeability of the apical membrane. Two different mechanisms present themselves as possible explanations for this activation, namely that the presence of Gd^{3+} in the apical solution might interfere with Na^+ self-inhibition and/or Na^+ feedback inhibition.

It is obvious that isolated epithelia from *Rana esculenta* display a pronounced degree of Na^+ self-inhibition (Fig. 5). In the presence of BIG in the apical solutions the Na^+ self-inhibition was abolished; thus, in the presence of a high apical Na^+-concentration, BIG keeps the apical P_{Na} in the high state (Fig. 5) (Zeiske & Lindemann, 1974; Garcia-Romeu, 1974). The presence of Gd^{3+} in the apical bathing solution apparently had no effect on the Na^+ self-inhibition but resulted in an activation of the I_{sc}.

The fact that BIG was able to abolish the activation by Gd^{3+} suggests that the two drugs at least in some way act via the same mechanism. Since it was also shown that Gd^{3+} only was capable of activating I_{sc} to 50% of the total activation seen when BIG was added, indicates that BIG has a binding site that it does not share with Gd^{3+}, probably being the Na^+ self-inhibition site.

This putative site of activation that they share might be due to an interaction of the two drugs per se with the Na^+ channel or it might be that they interfere with regulatory components that are integrated parts of the Na^+ feedback inhibition of the channel. Na^+ feedback inhibition is a process where changes in cellular Na^+ concentration, caused either by an increased entry rate or a decreased exit rate, indirectly

regulate the activity of the Na^+ channel (Turnheim, 1991). Several mechanisms have been proposed as the intermediate messengers of this inhibition: it has been shown that changes in the cellular Na^+ concentration due to changed transport rates of Na^+ can change intracellular pH (Lyall et al., 1994). Changes in intracellular Na^+ can be translated into $[Ca^{2+}]_i$ changes due to the existence of a basolateral Na^+-Ca^{2+} exchange mechanism (Madsen, Brodins & Nielsen, 1999). These changes can regulate the activity of the Na^+ channel (Harvey, Thomas & Ehrenfeld 1988; Brodin et al., 1996). Intracellular pH itself might act directly on the channel by protonation of certain amino acids and due to the effect on K^+ channels pH can alter the electrochemical driving force for Na^+ across the apical membrane (Harvey et al., 1988). Ca^{2+} has no direct effect on the Na^+ channel per se, but it possibly acts via protein kinases (Garty & Palmer, 1997). Another way of modulation of the channel has been shown to involve regulatory G-proteins that stimulate the action with a newly described cytosolic ubiquitin ligase Nedd4. Nedd4 binds to a proline-rich segment of the cytoplasmic carboxy-terminal part of the β and γ subunit of the ENaC, thereby controlling the turnover rate of the channel (Dinudom et al., 1998). Interestingly it has been shown that amiloride analogs, BIG among others, are capable of inhibiting this G-protein and thereby causing the whole-cell current to increase (Komwatana et al., 1998).

Since the uncharged form of amiloride is known to diffuse across membranes and considering the high similarity between BIG and amiloride, this might also be the case for BIG (Benos, Reyes & Shoemaker, 1983). Thus, the site of action that both BIG and Gd^{3+} act on might be a putative receptor for this G-protein. But the fact that there is no stimulatory effect when Gd^{3+} is presented to the basolateral side of the epithelium does not support this theory. The stimulation of Gd^{3+} is also present in the K^+- depolarized tissue, which is thought to be virtually empty of Na^+ (Fuchs et al., 1977) and therefore only downregulated to small degree, if at all, by the Na^+-feedback inhibition mechanism. If the G-protein is the site of action for the drugs, the G-protein has to be in a constitutive active mode in order for BIG and Gd^{3+} to inhibit its action. It is obvious that further evidence for this action is needed.

References

Benos, D.J., Reyes, J., Shoemaker, D.G. 1983. Amiloride fluxes across erythrocyte membranes. *Biochem. Biophys. Acta* **734:**99–104

Brodin, B., Nielsen, R. 2000. Evidence for P2Y-type ATP receptors on the serosal membrane of frog skin epithelium. *Pfluegers Arch.* **439:**234–239

Brodin, B., Rytved, K.A., Nielsen, R. 1996. An increase in $[Ca^{2+}]_i$ activates basolateral chloride channels and inhibits apical sodium channels in frog skin epithelium. *Pfluegers Arch.* **433:** 16–25

Dinudom, A., Harvey, K.F., Komwatana, P., Young, J.A., Kumar, S., Cook, D.I. 1998 Nedd4 mediates control of an epithelial Na^+ channel in salivary duct cells by cytosolic Na^+. *Proc. Natl. Acad. Sci. U.S.A.* **95:**7169–7173

Els, W.J., Helman, S.I. 1981. Vasopressin, theophylline, PGE2, and indomethacin on active Na transport in frog skin: studies with microelectrodes. *Am. J. Physiol.* **241:**F279–F288

Fuchs, W., Larsen, E.H., Lindemann, B. 1977. Current-voltage curve of sodium channel and concentration dependence of sodium permeability in frog skin. *J. Physiol.* **267:**137–166

Garcia-Romeu, F. 1974. Stimulation of sodium transport in frog skin by 2-imidazolines (guanidinbenzimidazole and phentolamine). *Life Sci.* **15:**539–542

Garty, H., Palmer, L.G. 1997. Epithelial sodium channels: function, structure, and regulation. *Physiol. Rev.* **77:**359–396

Gudme, C.N., Larsen, A.L., Nielsen, R. 2001. α_2-Adrenoceptors inhibit antidiuretic hormone-stimulated Na^+ absorption across tight epithelia (*Rana esculenta*). *Pfluegers Arch.* **442:**346–352

Harvey, B.J., Thomas, S.R., Ehrenfeld, J. 1988. Intracellular pH controls cell membrane Na^+ and K^+ conductances and transport in frog skin epithelium. *J. Gen. Physiol.* **92:**767–791

Helman, S.I., Cox, T.C., Van Driessche, W. 1983. Hormonal control of apical membrane Na transport in epithelia. Studies with fluctuation analysis. *J. Gen. Physiol.* **82:**201–220

Ismailov, I.I., Kieber-Emmons, T., Lin, C., Berdiev, B.K., Shlyonsky, V.G., Patton, H.K., Fuller, C.M., Worrell, R., Zuckerman, J.B., Sun, W., Eaton, D.C., Benos, D.J., Kleyman, T.R. 1997. Identification of an amiloride binding domain within the alpha-subunit of the epithelial Na^+ channel. *J. Biol. Chem.* **272:**21075–21083

Johnsen, A.H., Nielsen, R. 1978. Effects of the antidiuretic hormone, arginine vasotocin, theophylline, filipin and A23187 on cyclic AMP in isolated frog skin epithelium (*Rana temporaria*). *Acta Physiol. Scand.* **102:**281–289

Koefoed-Johnsen, V., Ussing, H.H. 1953. The contribution of diffusion and flow to the passage of D_2O through living membranes. *Acta Physiol. Scand.* **28:**60–76

Koefoed-Johnsen, V, Ussing, H.H. 1958. The nature of the frog skin potential. *Acta Physiol. Scand.* **42:**298–308

Komwatana, P., Dinudom, A., Young, J.A., Cook, D.I. 1998. Activators of epithelial Na^+ channels inhibit cytosolic feedback control. Evidence for the existence of a G protein-coupled receptor for cytosolic Na^+. *J. Membrane Biol.* **162:**225–232

Larsen, E.H., Ussing, H.H., Spring, K.R. 1987. Ion transport by mitochondria-rich cells in toad skin. *J. Membrane Biol.* **99:** 25–40

Li, J.H., Lindemann, B. 1983. Chemical stimulation of Na transport through amiloride-blockable channels of frog skin epithelium. *J. Membrane Biol.* **75:**179–192

Lindemann, B. 1984. Fluctuation analysis of sodium channels in epithelia. *Annu. Rev. Physiol.* **46:**497–515

Lyall, V., Belcher, T.S., Miller, J.H., Biber, T.U. 1994. Na^+ transport and pH in principal cells of frog skin: effect of antidiuretic hormone. *Am. J. Physiol.* **267:**R107–R114

Madsen, K.H., Brodin, B., Nielsen, R. 1999. Evidence for a Na^+ Ca^{2+} exchange mechanism in frog skin epithelium. *Pfluegers Arch.* **437:**175–181

Nagel, W., Garcia-Diaz, J.F., Essig, A. 1983. Cellular and paracellular conductance patterns in voltage-clamped frog skin. *Prog. Clin. Biol. Res.* **126:**221–231

Nielsen, R. 1979. 3 to 2 coupling of the Na–K pump responsible for the transepithelial Na transport in frog skin disclosed by the effect of Ba. *Acta Physiol. Scand.* **107:**189–191

Rytved, K.A., Brodin, B., Nielsen, R. 1995. Prostaglandin E2 release from dermis regulates sodium permeability of frog skin epithelium. *Acta Physiol. Scand.* **153:**263–270

Rytved, K.A., Nielsen, R. 1999. EP3 receptors inhibit antidiuretic-hormone-dependent sodium transport across frog skin epithelium. *Pfluegers Arch.* **437:**213–218

Sten-Knudsen, O., Ussing, H.H. 1981. The flux ratio equation under nonstationary conditions. *J. Membrane Biol.* **63:**233–242

Turnheim, K. 1991. Intrinsic regulation of apical sodium entry in epithelia. *Physiol. Rev.* **71:**429–445

Ussing, H.H. 1949. The distinction by means of tracers between active transport and diffusion. *Acta Physiol. Scand.* **19:**43–56

Ussing, H.H., Zerahn, K. 1951. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta Physiol. Scand.* **23:**109–127

Zeiske, W, Lindemann, B. 1974. Chemical stimulation of Na^+ current through the outer surface of frog skin epithelium. *Biochim. Biophys. Acta* **352:**323–326