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Prostaglandin E_2 Enhances the Sodium Conductance of Exocrine Glands in Isolated Frog Skin (*Rana esculenta*)

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Summary. Prostaglandins are known to stimulate the active transepithelial Na^+ uptake and the active secretion of Cl^- from the glands of isolated frog skin. In the present work the effect of prostaglandin E_2 (PGE_2) on the glandular Na^+ conductance was examined. In order to avoid interference from the Na^+ uptake and the glandular Cl^- secretion the experiments were carried out on skins where the Cl^- secretion was inhibited (the skins were bathed in Cl^- Ringer's solution in the presence of furosemide, or in NO_3^- Ringer's solution), and the active Na^+ uptake was blocked by the addition of amiloride. Transepithelial current, water flow and ion fluxes were measured. A negative current was passed across the skins (the skins were clamped at -100 mV, basolateral solution was taken as reference). When PGE_2 was added to the skins under these experimental conditions, the current became more negative; this was mainly due to an increase in the Na^+ efflux. Together with the increase in Na^+ efflux a significant increase of the water secretion was observed. The water secretion was coupled to the efflux of Na^+, and when one Na^+ was pulled from the basolateral to the apical solution via this pathway 230 molecules of water followed. From the data presented it is suggested that this pathway for Na^+ is confined to the exocrine glands.

Key Words Electro-osmosis · frog skin glands · local osmosis · prostaglandin E_2 · sodium conductance

Introduction

The isolated frog skin consists of at least three different types of cells which are engaged in transepithelial ion and water transports; these are: the granular cells, the mitochondria-rich cells and the glandular cells. For a more detailed description of the histology of frog skin epithelia and glands, *see* Voûte (1963), Farquhar and Palade (1965), Mills and Prum (1984). The granular cells (*stratum granulosum, -spinosum* and *-germinativum*), which make up the major part of the epithelium, are responsible for the active transepithelial Na^+ uptake, whereas the mitochondria-rich cells (which are scattered in the epithelium) form the passive (potential-activated) pathway for Cl^- (for references, *see* Kristensen & Ussing, 1985; Larsen, 1988). The acinus of the exocrine glands of frog skin is located in the dermis, and the glandular duct crosses the epithelial layer.

The exocrine glands of frog skin can be stimulated to secrete, both in vivo and in vitro (Watlington, 1968; Benson & Hadley, 1969; Skoglund & Sjöberg, 1977*a,b*). The initial secretion pulse involves a transient bulk expulsion of the contents of the acinus by contraction of smooth muscle or myoepithelial cells which surround the gland acini (Benson & Hadley, 1969; Hoffman & Dent, 1977). This expulsion is mediated via α-adrenergic receptors and is not characterized by an increase in transepithelial current or ion transport (Watlington, 1968). The continuous secretion of fluid onto the skin surface, which follows after the initial secretion pulse, is driven by an active secretion of Cl^- and is stimulated via β-adrenergic receptors (Watlington & Huf, 1971; Thompson & Mills, 1981, 1983). The active secretion of Cl^- can be measured as an increase in the short-circuit current (SCC) across isolated frog skin (Koefoed-Johnsen, Ussing & Zerahn, 1952; Watlington, 1968; Tomlinson & Wood, 1978; Thompson & Mills, 1981, 1983; Bjerregaard & Nielsen, 1987). This secretion of Cl^- can be explained by the model proposed by Silva et al. (1977). According to the model, glandular Cl^- secretion occurs as follows: Cl^- and Na^+ enter the glandular cells from the blood side via a cotransport mechanism (NaCl or NaK2Cl). The gradient for inward movement of NaCl into the cells is created by the Na-K pump. Secretory agents are believed to increase, directly or indirectly, the Cl^- permeability of the apical membrane of the gland cells. Cl^- diffuses from the cells to the lumen down a favorable electrochemical gradient. This secretion of Cl^- makes the glandular lumen negative relative to the blood side of the glands, which drags Na^+ from the blood side of the gland to the lumen. Thus, the

model predicts that glandular secretion of NaCl can be modified either by changing the rate of Cl^- secretion or by changing the transglandular Na^+ conductance. In a previous paper (Bjerregaard & Nielsen, 1987) it is shown that PGE_2 activates the secretion of Cl^- from the skin glands. The aim of the present paper is to investigate whether PGE_2 should have an effect on the glandular Na^+ conductance.

From the data presented it is seen that PGE_2 activates the Na^+ conductance of the glands, and when Na^+ is dragged across the glands (from the basolateral to the apical solution) by an external applied potential, each Na^+ is accompanied by about 230 molecules of water. A preliminary report of these results has been presented (Nielsen, 1988).

Materials and Methods

The experiments were performed on male and female frogs (*Rana esculenta*), which were kept at room temperature with free access to water; they were fed twice a week with meal worms.

The isolated abdominal skins were divided into two symmetrical halves so each skin served as its own control. The skins were mounted in Perspex® chambers (area 8 cm^2) and bathed in a stirred Cl^- Ringer's solution consisting of (in mM): Na^+ 115, K^+ 2.5, Ca^{2+} 1, Mg^{2+} 1, Cl^- 118, CO_3^{2-} 2.5, PO_4^{3-} 1, pH = 7.8.

Nitrate Ringer's solution was produced by replacing all Cl^- by NO_3^-.

Flux Measurements

In ion flux experiments one skin half was used for influx measurements and the other for efflux measurements. $^{22}Na^+$ and $^{36}Cl^-$ were added to the solution, bathing one side of the skins. After a 20-min equilibration period, a 1-ml aliquot was withdrawn from the other side and replaced by fresh solution. The last procedure was repeated with 30- or 60-min intervals throughout the experiment, under conditions where the bathing solutions were continuously stirred.

Volume Flow

SCC and volume flow (Jv) were measured simultaneously under conditions where the skin was bathed with isotonic Ringer's solution on both sides. Jv was measured as described by Johnsen and Nielsen (1980) by an improved technique which allowed recordings of Jv at intervals of 1 min. In short, the principle is that the outside of the skins is pressed against a stainless steel net with a pressure of 2 cm H_2O. The half-chamber (area: 8 cm^2), containing the outside bathing solution is closed except for an outlet consisting of a capillary tube. The outside bathing solution is allowed to flow into the capillary tube, whereby the light transmission of the tube changes markedly. The transmission is recorded by a detector consisting of a light-emitting diode and a photosensitive transistor. The signal from the detector controls a motor-driven syringe which appropriately adjusts the outside volume in order to keep the position of the meniscus constant. The motor also drives a precision potentiometer which is used as a potential divider, allowing the position of the syringe to be recorded continuously on a pen recorder.

Results

The aim of the present investigation was to examine whether PGE_2 increases the Na^+ conductance of exocrine glands present in isolated frog skin. It is well known that PGE_2 enhances the Na^+ conductance of the granular cells (Barry & Hall 1969; Fassina, Carpenedo & Santi, 1969; Barry, Hall & Martin, 1975; Gerencser, 1978). Therefore, in order to avoid interference from the Na^+ conductance of these cells, the Na^+ channels in the apical membrane were blocked by the addition of amiloride, and in order to the reduce the interference from the active Cl^- secretion from the glands the cotransport inhibitor furosemide was added, or the Cl^- Ringer's solution was replaced by NO_3^- Ringer's solution.

Effect of Cotransport Inhibitors

In Fig. 1 an experiment is presented where both skin halves were bathed in Cl^- Ringer's solution in the presence of amiloride, and furosemide was added to one of the skin halves. The short circuit current and the transepithelial volume flow (which at physiological concentrations is equal to the water flow) were measured. The addition of PGE_2 resulted initially in a transient increase in the water secretion in both skin halves. In the skin half incubated in the absence of furosemide the water secretion returned to a new steady level after the initial secretion pulse had ceased, whereas the secretion stopped in the skin half incubated in the presence of furosemide. The initial transient increase in the water secretion is probably, as mentioned in the introduction, due to a PGE_2-induced activation of the myoepithelial cells or smooth muscles surrounding the glands. In the absence of furosemide PGE_2 elicited a significant increase in SCC, but in the presence of furosemide the effect of PGE_2 on SCC was much less pronounced. The observed increase in SCC (in the absence of furosemide) is mainly due to an activation of the glandular secretion of Cl^- (Bjerregaard & Nielsen, 1987). If instead of using the cotransport inhibitor furosemide, the Cl^- Ringer's solution was replaced by NO_3^- Ringer's solution. The result was qualitatively the same as the one obtained in Cl^- Ringer's solution in the presence of furosemide (*data not shown*).

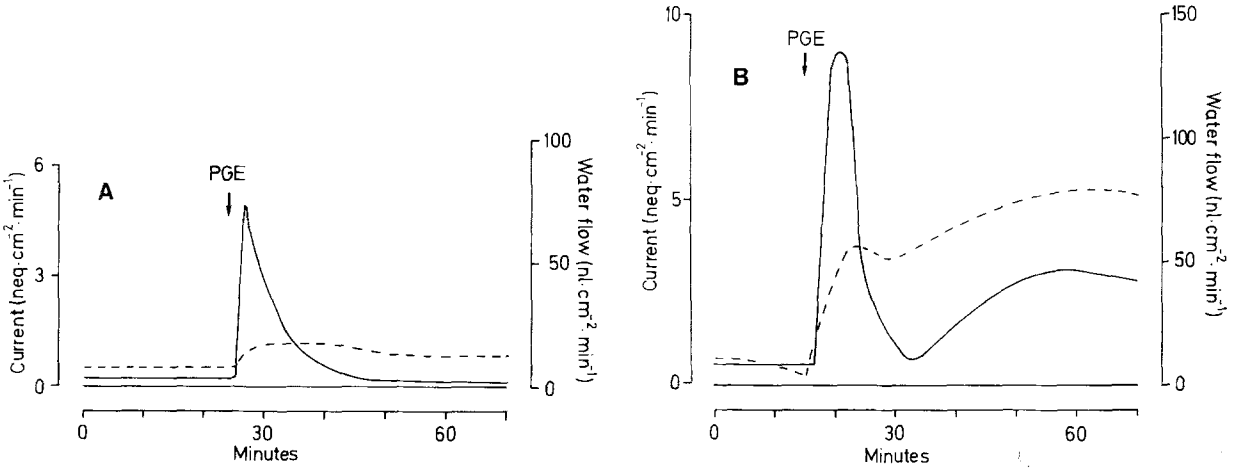

Fig. 1. (*A*) Transepithelial short circuit current (broken line) and water flow (solid line) across isolated frog skin. Positive water flow corresponds to water secretion. Positive current corresponds to an uptake of a positive ion or the secretion of a negative ion. The skin was bathed in Cl^- Ringer's solution in the presence of amiloride (0.1 mM apical) and furosemide (0.5 mM basolateral). At the arrow labeled *PGE*, PGE_2 was added (2 μM basolateral). (*B*) Control skin half, incubated under the conditions mentioned in *A*, but in the absence of furosemide

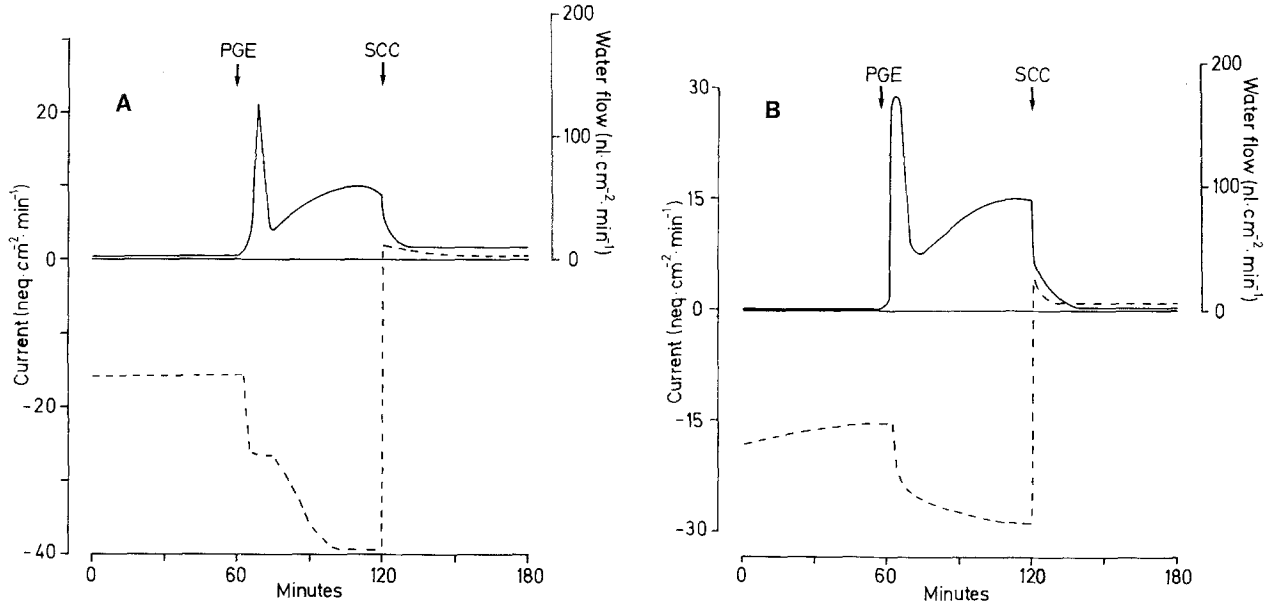

Fig. 2. (*A*) Transepithelial current (broken line) and water flow (solid line) across an isolated frog skin bathed in NO_3^- Ringer's solution in the presence of amiloride (0.1 mM apical). The skin was clamped at −100 mV (basolateral solution taken as reference) for the first 120 min; at the arrow labeled *SCC* the skin was clamped at 0 mV (short-circuited conditions). At the arrow labeled *PGE*, PGE_2 was added (2 μM basolateral). A negative current corresponds to a secretion of a positive ion or an uptake of a negative ion. (*B*) The experiment was carried out as described in the legend to *A*, but the skin was bathed in Cl^- Ringer's solution in the presence of amiloride (0.1 mM apical) and furosemide (0.1 mM basolateral)

Conductance

In order to investigate whether PGE_2 had an effect on the transglandular Na^+ conductance, experiments of the type presented in Fig. 2 were carried out. One of the skin halves was incubated in NO_3^- Ringer's solution (Fig. 2*A*) and the other half in Cl^- Ringer's solution in the presence of furosemide (Fig. 2*B*). Amiloride was added to both skin halves and the skins were clamped at −100 mV (basolat-

Table. The effect of PGE_2 on transepithelial current, water flow, Na^+ and Cl^- effluxes across isolated frog skins clamped at −100 mV

Conditions	*n*	Current neq/cm²/min	Water-flow nl/cm²/min	Na^+ efflux neq/cm²/min	Cl^- efflux neq/cm²/min
Control	6	−30.8 ± 7.3	6.6 ± 3.7[a]	12.3 ± 3.1	1.60 ± 0.36
Control + PGE		−39.4 ± 5.8	71.0 ± 4.4	23.3 ± 2.5	6.97 ± 0.33
Difference		−8.6 ± 0.7	64.9 ± 4.2	10.9 ± 0.7	5.38 ± 0.44
0.5 mM Furos	6	−35.5 ± 9.7	10.3 ± 8.1[a]	15.4 ± 4.7	1.92 ± 0.53
0.5 mM Furos + 0.5 mM Furos + PGE		−38.7 ± 9.2	20.4 ± 6.6	18.6 ± 4.5	1.81 ± 0.35
Difference		−3.2 ± 0.7	10.2 ± 3.2	3.2 ± 0.7	−0.11 ± 0.21
0.1 mM Furos	5	−10.5 ± 2.1	−11.3 ± 2.6[a]	2.9 ± 0.8	0.86 ± 0.16
0.1 Furos + PGE		−16.3 ± 3.5	20.7 ± 9.3	8.9 ± 2.4	1.40 ± 0.23
Difference		−5.8 ± 2.0	32.6 ± 10.1	6.0 ± 2.0	0.55 ± 0.19
NO_3^- solution	7	−11.3 ± 1.2	1.2 ± 4.1[a]	5.6 ± 0.6	
NO_3^- solution + PGE		−20.8 ± 2.6	34.0 ± 9.2	14.8 ± 2.4	
Difference		−9.5 ± 1.9	32.8 ± 7.5	9.3 ± 1.8	

The experiments were carried out in the presence of amiloride (0.1 mM apical) in Cl^- Ringer's solution except for the last series which were carried out in NO_3^- Ringer's solution. Furosemide (Furos) was present in two of the series 0.5 or 0.1 mM (basolateral). The mean of the current, water flow and ion fluxes were measured during a 1-hr period just before the addition of PGE_2 (PGE) and during another 1-hr period 30 min after the addition of PGE_2 (2 μM basolateral). the data are the mean ± SE.

[a] $n = 4$. A negative current corresponds to the secretion of a positive ion or an uptake of a negative ion. Positive water flow corresponds to water secretion.

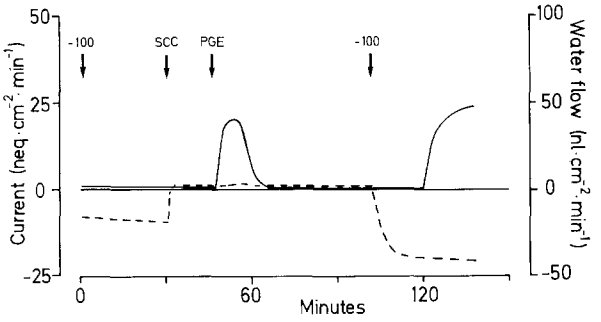

Fig. 3. Current (broken line) and transepithelial water flow (solid line) across isolated frog skin bathed in NO_3^- Ringer's solution in the presence of amiloride (0.1 mM apical). Initially the skin was clamped at −100 mV (basolateral solution taken as reference). At the arrow labeled SCC the skin was clamped at 0 mV. At the arrow labeled PGE, PGE_2 was added (2 μM basolateral). After about 100 min of incubation the skin was clamped at −100 mV (at the arrow labeled *−100*)

eral solution reference), and the transepithelial current and water secretion were measured. When PGE_2 was added to the skin halves under these conditions the current became more negative and there was a transient increase in the water secretion as shown above. But after the transient increase, the water secretion continued (although the cotransport system was inhibited) as long as a current was dragged across the skins. When the clamp voltage was changed from −100 to 0 mV (short-circuited conditions) the current changed from the relatively high negative level to a slightly positive level and the water secretion ceased. In Fig. 3 an experiment is shown which is analogous to the one presented in Fig. 2*A*, but in Fig. 3 PGE_2 was added under short-circuited conditions, and thereafter the skin was clamped at −100 mV, but not until the initial secretion pulse had ceased completely. Clamping of the skin at −100 mV after the addition of PGE_2 resulted immediately in the current becoming negative, but the water secretion did not start until 12.7 ± 2.2 min ($n = 6$) after the current had been turned on. Thus it looks as if there is a sort of pool (e.g., the glandular lumen) which has to be filled before the onset of the water secretion. Since the current became more negative after the addition of PGE_2 then either the inward-directed anion flow or the outward-directed cation flow has to increase, and since the water secretion did increase too, it is most likely that the change in current is mainly due to an increase of the Na^+ efflux. That this is so appears from the data presented below.

Effect of PGE_2 On Ion Flux

In the Table experiments are presented in which the $^{22}Na^+$ and $^{36}Cl^-$ efflux were measured across the skins before and after addition of PGE_2. The skins were clamped at −100 mV and amiloride was present in the apical bathing solution. The fluxes

were measured during a 1-hr period just before the addition of PGE_2 and during another 1-hr period 30 min after the addition of PGE_2. The 30-min interval between the flux measurements was included in order to avoid interference from the α-like stimulation of the glands. The first two rows of data in the Table contain data obtained on symmetrical skin halves incubated under the conditions mentioned above. One of the skin halves was incubated in the presence of furosemide (0.5 mM) and the others in the absence of furosemide. In the absence of furosemide the addition of PGE_2 resulted in an increase of the Na^+ efflux of 10.9 neq cm^{-2} min^{-1}, and the increase in Cl^- efflux was 5.4 neq cm^{-2} min^{-1}. In the presence of furosemide (0.5 mM) PGE_2 had no effect on the Cl^- efflux, but the Na^+ efflux did increase by 3.2 neq cm^{-2} min^{-1}. In the presence of 0.1 mM furosemide the increase in the Cl^- efflux was 0.55 neq cm^{-2} min^{-1} and the increase in the Na^+ efflux was 6.0 neq cm^{-2} min^{-1} (*see* Table). Thus the presence of furosemide blocked the PGE_2-induced increase in the Cl^- efflux but not the PGE_2-induced increase in the Na^+ efflux. When the skin halves were bathed in NO_3^- Ringer's solution as well as in Cl^- Ringer's solution (in the presence of furosemide) the change in Na^+ efflux, after the addition of PGE_2, was nearly equal to the observed change in current (*see* Table). Since, under these conditions the PGE_2-induced change in $^{22}Na^+$ influx was small (in NO_3^- Ringer's solution the Na^+ influx changed from 0.7 ± 0.3 to 0.4 ± 0.1 (n = 5) neq cm^{-2} min^{-1} and in Cl^- Ringer's solution from 0.2 ± 0.1 to 0.6 ± 0.1 neq cm^{-2} min^{-1} (n = 7), then the change in the net flux of anions has to be small. Therefore the observed changes in current are mainly due to the change in Na^+ efflux. Thus PGE_2 enhances the transepithelial Na^+ conductance of the isolated frog skin incubated under these conditions. The discrepancy between the current and the ion fluxes both before and after the addition of PGE_2 (*see* Table) is due to an inward-directed flux of anions.

Osmolarity

The transepithelial water movement across skins bathed either in NO_3^- Ringer's solution or in Cl^- Ringer's solution in the presence of furosemide was low, even though a current was dragged across the skins (the skins were clamped at −100 mV). When PGE_2 was added to skins (bathed in NO_3^- or Cl^- Ringer's solution in the presence of furosemide) the transepithelial current became more negative and the water secretion increased. The change in current was mainly due to an increase in the Na^+ efflux (*see* Table). Since the basal current and the PGE_2-induced changes in Na^+ and water secretion varied from skin to skin, the apparent Na^+ concentration of the solution, which moves across the skin bathed in NO_3^- Ringer's solution, was obtained by plotting the PGE_2-induced increase in Na^+ efflux *versus* the increase in water secretion (Fig. 4). The slope of the regression line was 0.240 which corresponds to an apparent Na^+ concentration of 240 mM, or in other words when one Na^+ was dragged across the skin from the basolateral to the apical solution via the PGE_2-induced pathway then about 230 water molecules followed. The PGE_2-induced increase in (Na^+ + Cl^-) effluxes (circles) and the increase in the Na^+ efflux (triangles) of skins bathed in Cl^- Ringer's solution in the presence of furosemide is plotted as a function of a concomitant increase in the water secretion (Fig. 5). The slope of the regression line, fitted to the increase in (Na^+ + Cl^-) effluxes was 0.208. The difference in position between the circles and the triangles is small, which is in agreement with the fact that the PGE_2-induced increase in the Cl^- efflux, in the presence of furosemide, is small. Thus, under conditions where the cotransport system is blocked, the water secretion is coupled, either directly or indirectly, to the efflux of Na^+ which is dragged across the skins via a PGE_2-induced pathway.

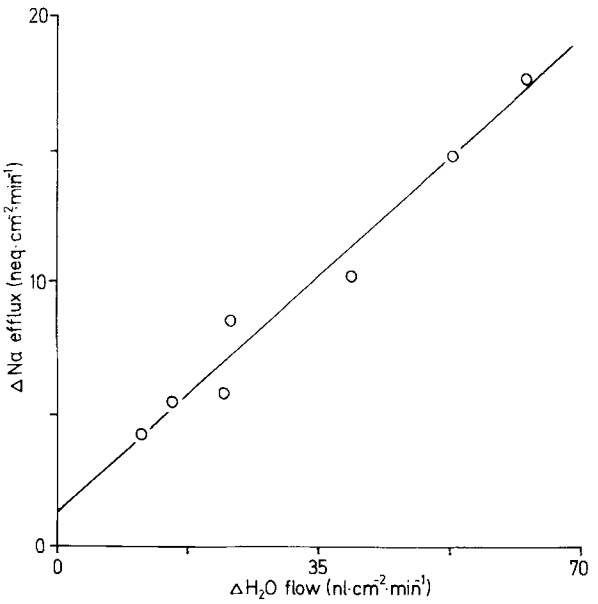

Fig. 4. PGE_2-induced increase in the Na^+ efflux plotted as function of the PGE_2-induced increase in the water secretion. The skins were bathed in NO_3^- Ringer's solution in the presence of amiloride (0.1 mM apical) and were clamped at −100 mV. The linear regression line was drawn according to the following equation: $y = 1.43 + 0.24\ x$, where y is equal to the PGE_2-induced increase in the Na^+ efflux and x is equal to the PGE_2-induced increase in the water secretion

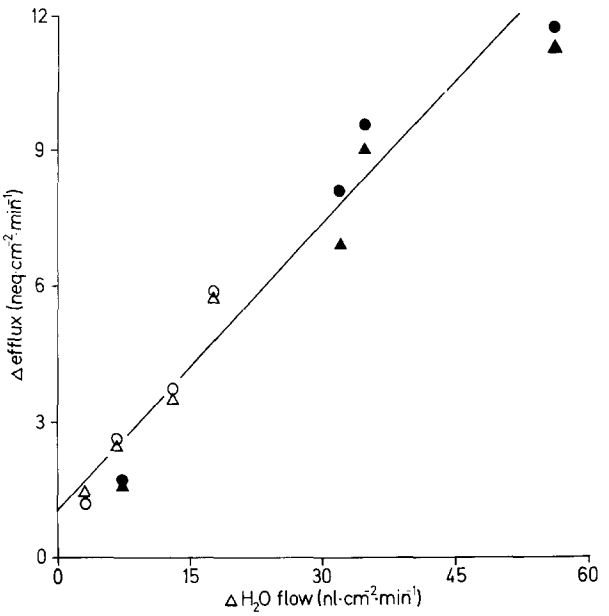

Fig. 5. PGE_2-induced increase in transepithelial (Na^+ + Cl^-) efflux (circles), and PGE_2 increase in transepithelial Na^+ efflux (triangles) plotted as function of the PGE_2-induced increase in the water secretion. The experiments were carried out in Cl^- Ringer's solution in the presence of amiloride (0.1 mM apical) and furosemide (basolateral); open symbols 0.5 mM furosemide, filled symbols 0.1 mM furosemide. The skins were clamped at −100 mV. The linear regression line was drawn according to the following equation: $y = 1.01 + 0.208\ x$, where y is equal to the PGE_2-induced increase in the (Na^+ + Cl^-) efflux and x is equal to the PGE_2-induced increase in the water secretion. The PGE_2-induced increase the Na^+ efflux as function of the water secretion could be described by the following equation: $y = 1.13 + 0.192\ x$

Discussion

From the data presented in this paper it is seen that activation of the glands of short-circuited skins (PGE_2 was added to the basolateral solution), resulted initially in a transient secretion, whether the skins were bathed in Cl^- or NO_3^- Ringer's solution (Fig. 1). This initial secretion is probably due to the fact that PGE_2 stimulate the smooth muscle or myoepithelial cells surrounding the gland acini (Benson & Hadley, 1969; Hoffman & Dent, 1977), a stimulation which results in a bulk secretion by a rapid expulsion of material contained in the lumen of the gland (Watlington, 1968).

After the initial secretion pulse the glands of short-circuited skins bathed in Cl^- Ringer's solution continued to secrete, but not if the skins were bathed in NO_3^- Ringer's solution or in Cl^- Ringer's solution, in the presence of furosemide (Fig. 1). It has been shown previously that NO_3^- is a bad substitute for Cl^- in glandular secretion (Lundberg, 1956; Seldin & Hoshiko, 1966; Petersen, 1988). This is probably due to the fact that the active transport (secretion) of anions requires an active cotransport system (Silva et al., 1977), and NO_3^- cannot substitute for Cl^- in the cotransport system (Kinne, 1988).

When frog skins bathed in NO_3^- ringer's solution or Cl^- Ringer's solution, in the presence of furosemide, were clamped at −100 mV, the transepithelial current was carried partially by a net efflux of Na^+ and a net influx of anions (*see* Table). Under these conditions addition of PGE_2 had the result that the transepithelial current became more negative. This change in current was mainly due to an increase in the efflux of Na^+. Thus PGE_2 enhances the Na^+ conductance of the skins. The observed increase in the Na^+ efflux was followed by an increase in the secretion of water, and there was a linear correlation between the increase in Na^+ efflux and water secretion (Figs. 4 and 5), which indicates that the water secretion is coupled to the PGE_2-induced increase in Na^+ efflux. From the considerations presented below it appears that it is most likely that this coupling between the Na^+ efflux and the water secretion takes place in the glands.

Water Flow

Water flow across membranes has been investigated theoretically by several authors (Staverman, 1952; Lorimer, Boterenbrood & Hermans, 1956; Kedem & Katchalsky, 1963). Water flow across a membrane may consist of the following three components: water flow = hydraulic flow + osmotic flow + electro-osmotic flow, (House, 1974). Thus, three different types of forces can drive water across a membrane, i.e., hydrostatic pressure gradient, osmotic pressure gradient and electrical potential gradient. Transmembrane water flow, which arises as a result of ions being dragged across the membrane by an electrical force, may be due to electro-osmosis (Spiegler, 1958; Dainty, Croghan & Fensom, 1963; Hill, 1975) or to current-induced changes in the osmolarity of the solution bathing the membrane (local osmosis) (Barry & Hope, 1969*a*,*b*). The occurrence of local osmosis requires a membrane area where there is a relatively great difference between the cation and anion permeability (e.g., an area which is permeable for Na^+ but impermeable for NO_3^-), given that Na^+ and NO_3^- have comparable transport number values in the adjacent solution.

Local Osmosis

According to Barry and Hope (1969*a*,*b*), the passage of a current via a membrane with such a difference in transport numbers would (because of the presence of unstirred layers) result in a decrease in the concentration of $NaNO_3$ at the membrane solution interface from which the Na^+ is removed. On the other hand, the $NaNO_3$ concentration would increase in the membrane solution interface in the phase to which Na^+ is moved. If such a local osmotic gradient should result in something like an iso-osmotic transport, it would require that the effective water permeability of the membrane was much higher than the effective water permeability of the unstirred layer. This may be the case in frog skin glands, because the area of the gland, in connection with the basolateral solution, is much larger than the area in connection with the apical solution (the opening of the glandular duct). Furthermore the frog skin epithelium is a high resistance epithelium, whereas the glandular epithelium, except for the glandular duct, is classified as a low resistance epithelium. If the water secretion is caused by local osmosis it is very likely that the observed PGE_2-induced increase in Na^+ efflux is confined to the glands.

However if the observed water secretion was due to electro-osmosis it would require an electro-osmotic element somewhere in the skin.

Electro-Osmosis

Spiegler (1958), Dainty et al. (1963) and Kedem (1965) have analyzed electro-osmosis in terms of frictional coefficients between water, ion and membrane excluding co-ions (because of the Donnan exclusion). The ratio of water flow to ion flow, leaving an electro-osmotic element, is given by Eq. (1) (Spiegler, 1958; Dainty et al., 1963).

Mole of water electro-osmotic transported per mole of ions

$$\frac{f_{13}}{(C1/C3)f_{13} + f_{34}}. \qquad (1)$$

Equation (1) is based on the assumption that the volume flow is equal to the water flow, which is correct at physiological concentrations. $C1$ and $C3$ are the concentrations of Na^+ and water in the electro-osmotic element. f_{13} and f_{34} are the molar frictional coefficients between water and ion (Na^+) and between water and membrane, respectively. According to the relative magnitude of f_{13} and f_{34} it is apparent that, when the water-membrane friction is low compared to the water-solute friction, the concentration of the transported fluid becomes equal to the concentration in the element.

The data presented show that when one Na^+ ion is dragged across the skin glands (from the basolateral to the apical solution by an imposed potential difference) then 230 molecules of water follow. If the water secretion was due to electro-osmosis then the electro-osmotic component (the water secretion) should reach a maximum immediately after the current had been turned on (Barry & Hope, 1969*b*). From Fig. 3 it is seen that if the current was turned on, after the α-like stimulation has ceased, there was a considerable delay between the increase in current and water flow. Therefore the observed change in water flow is either not caused by electro-osmosis or, if it is caused by electro-osmosis, the electro-osmotic element must be placed in an area of the skin where a pool has to be filled before the secretion starts. The only reasonable place where this pool can be placed is in the lumen of the glands. Therefore, whether the water secretion is caused by electro-osmosis or local osmosis or a combination of these, the only reasonable place where this can taken place is in the glands. Consequently, it is concluded that the observed PGE_2-induced increase in Na^+ efflux is confined to the glands.

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