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Papaverine Reduces the Sodium Permeability of the Apical Membrane and the Potassium Permeability of the Basolateral Membrane in Isolated Frog Skin

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Summary. The effect of papaverine, an inhibitor of the phosphodiesterase responsible for breakdown of cAMP, on the transepithelial sodium transport across the isolated frog skin was investigated.

Serosal addition of papaverine caused initially an increase in the short-circuit current (SCC), a doubling of the cellular cAMP content and a depolarization of the intracellular potential under SCC conditions (V_{scc}).

The initial increase in the SCC was followed by a pronounced decrease both in the SCC and in the natriferic action of antidiuretic hormone (ADH), but papaverine had no inhibitory effect on the ability of ADH to increase the cellular cAMP content. As SCC declines, no hyperpolarization was observed.

The I/V relationship across the apical membrane during the inhibitory phase, revealed that papaverine reduces the sodium permeability of the apical membrane (P^a_{Na}) as well as intracellular sodium concentration. These observations and the previously noted effect of papaverine on V_{scc} indicates that papaverine must have an effect on the cellular Cl or K permeability.

The basolateral Na,K,2Cl cotransporter was blocked with bumetanide, which should bring the cellular chloride in equilibrium. Bumetanide had no effect on basal SCC and V_{scc}. When papaverine was added to skins preincubated with bumetanide, the effect of papaverine on SCC and V_{scc} was unchanged. Therefore, the depolarization of V_{scc}, observed during the papaverine-induced inhibition of the SCC, must be due to a reduction in the cellular K permeability.

In conclusion, it is suggested that papaverine reduces the sodium permeability of the apical membrane and the potassium permeability of the basolateral membrane of the frog skin epithelium.

Key Words cAMP · cell Na^+ · frog skin · Na^+ permeability · papaverine

Introduction

The reported effects of the opiate derivative papaverine show great diversity. Papaverine is known to block phosphodiesterases and to increase both cyclic adenosine 3′,5′-monophosphate (cAMP) and cyclic guanosine 3′,5′-monophosphate (cGMP) level in cells (Lugnier & Stocklet, 1974; Miyamoto et al., 1976).

Papaverine is also a nonspecific smooth muscle relaxant, thought to act at a site beyond the receptor sites on the cell membrane (Hertle & Nawrath, 1990), which could be due to an inhibition of calcium channels in the cell membrane (Bolton, 1979).

Experiments from this laboratory, where the effects of papaverine on active sodium transport in frog skin epithelium were examined (Johnsen & Nielsen, 1984), have revealed that papaverine shows properties which could not be entirely explained as an inhibition of the phosphodiesterase responsible for breakdown of cAMP.

The purpose of this study was to determine the effects of papaverine on active sodium transport.

The experiments showed that papaverine added to the serosal solution of the frog skin resulted in a decrease of the sodium permeability in the apical membrane. It also decreased the sodium concentration in the granular cells without affecting the Na/K pump in the basolateral membrane.

The results further suggest that papaverine decreases the potassium permeability in the basolateral membrane of the frog skin epithelium. This could be due to interactions with calcium, since papaverine is a calcium antagonist, and the existence of potassium channels sensitive to calcium has recently been demonstrated in the basolateral membrane of the frog skin epithelium (Harvey, Urbach & Van Kerkhove, 1991).

Materials and Methods

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

All data in this paper are mean ± SE unless otherwise noted.

V_{scc} Measurements

The intracellular potential, V_{scc}, was measured under short-circuited conditions as described by Helman and Fisher (1977). Microelectrodes for V_{scc} measurements were prepared from single-barreled glass capillaries (GC 120 F-10, Clark Electromedical Instruments, UK) drawn into micropipettes on a puller (BB-CH Mecanex, Switzerland) and backfilled with 0.3 M KCl. The epithelium was mounted in Perspex chambers with the serosal side exposed to constant flowing Ringer's solution and the mucosal side facing a well-stirred Ringer's solution. The microelectrode was advanced across the apical membrane of the epithelium by means of a stepping motor-driven micromanipulator. The electrode resistance (R_E) was monitored throughout the experiment and was typically 100–150 MΩ. Minimal criteria for acceptability of impalements were: stability for several minutes, identical values for R_E before and after penetration, and finally, a negligible offset when the electrode is withdrawn.

During impalements of the short-circuited skin the command voltage was changed to ±10 mV, and from the voltage-divider ratio, the fractional resistance of the apical membrane (fR_a) was determined from the equation

$$fR_a = \frac{R_a}{R_a + R_b} = \frac{\delta V_a}{\delta V_t} \quad (1)$$

where R_a and R_b are the resistances of the apical and basolateral membrane, respectively, δV_a is the PD obtained when the command voltage was changed from +10 to −10 mV; δV_t = 20 mV.

When appropriate, the I/V relationship across the apical membrane was measured as described by Schoen and Erlij (1985). To measure the I/V relationship a series of nine pairs of pulses in subsequent steps (magnitude 20 mV, duration 250 mSec, hyperpolarization and depolarization, respectively, interpulse duration 8 sec) was performed. A computer was programmed to deliver the pulses and to sample the current and the potential from the experimental apparatus. During a pulse sequence V_{scc} has to return to the initial value in the interpulse phase in order to confirm the impalement. During each pulse value the apical membrane potential, PD_a, and the transepithelial current, I_T, was measured. After the I/V relationship was measured, 50 μM amiloride was added the mucosal bathing solution, and subsequently, the I/V relationship in the presence of amiloride was measured to obtain the amiloride-sensitive current, I_{am}.

From the data, the I_{am} from each pulse value was subtracted from the respective value of I_T in the absence of amiloride in order to obtain the net cellular current across the apical membrane. The amiloride-sensitive current is the Na current, since the general assumption is that the apical membrane is exclusively Na selective (Koefoed-Johnsen & Ussing, 1958).

SCC Measurements

The SCC measurements were performed by the method described by Ussing and Zerahn (1951). Symmetrical abdominal skin halves were mounted in Perspex chambers, and the transepithelial voltage was clamped at 0 mV. The SCC is considered positive when the current is directed inward, that is the flux of a positive ion from the apical side to the basolateral side. The skins were bathed in Ringer's solution of the following composition (all values in mM): Na^+ 115, K^+ 2.5, Ca^{2+} 1, Mg^{2+} 1, Cl^- 118, HCO_3^- 2.5, $H_2PO_4^-$ 1, glucose 5, pH = 7.8. The experiments were performed at room temperature.

In order to determine the skin resistance during the experiments, the skins were momentarily clamped at ±10 mV for approximately 5 sec.

In flux experiments, one skin half was used for efflux measurements and the other for influx measurements. $^{22}Na^+$ was 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 with fresh solution. The last procedure was repeated at 30-min intervals throughout the experiment. $^{22}Na^+$ activity was assayed in a Packard liquid scintillation counter.

cAMP Measurements

The abdominal skin was divided into four sections, each of which was divided into two symmetrical halves. The skin pieces were transferred to test tubes containing collagenase solution (0.3 mg/ml Ringer's solution). After incubation in the collagenase solution the epithelium was removed from the skin and transferred to fresh medium. After 1-hr incubation the isolated epithelium was exposed to experimental conditions.

The cAMP was extracted by dropping the epithelium into a test tube containing 500 μl distilled water (100°C), which also contained ^3H-cAMP as a recovery marker. The test tube was capped and after 5 min mixed on a Vortex mixer and placed in an ice bath. The tubes were centrifuged 10 min at 10,000 × g. The supernatant was placed on approximately 1.4 g dry Al_2O_3 in a minicolumn (Evergreen Scientific, UK) and eluted with 5 ml distilled water. The eluate was lyophilized and dissolved in a buffer, and cAMP was measured by a binding protein assay (Geisler et al., 1977). The overall recovery was typically 70%. The precipitate was dried at 70°C, 50 mm Hg, and the dry weight was determined.

Chemicals

Collagenase (from *Clostridium histolyticum*) was from Boehringer Mannheim; amiloride, arginine vasotocin (AVT) and papaverine was from Sigma Chemical, St. Louis, MO. Bumetanide was a gift from Leo Pharmaceuticals, Ballerup, Denmark.

Results

Addition of papaverine (150 μM) to the basolateral solution resulted as shown in Fig. 1 in a stimulation of the SCC.

The control level was 17.3 ± 2.1 μA cm^{-2}, and after 20-min incubation with papaverine, the SCC was 23.0 ± 2.9 μA cm^{-2}. After the initial stimulation the SCC started to decrease. After 60-min incubation with papaverine the SCC was 8.8 ± 2.2 μA cm^{-2}. Addition of the antidiuretic hormone, AVT (48 nM), normally stimulates the production of cAMP and thereby the SCC. When added to skin halves preincubated with papaverine only a small activation of the SCC was observed (Fig. 1). The reduction of the natriferic action of AVT depends on the concentra-

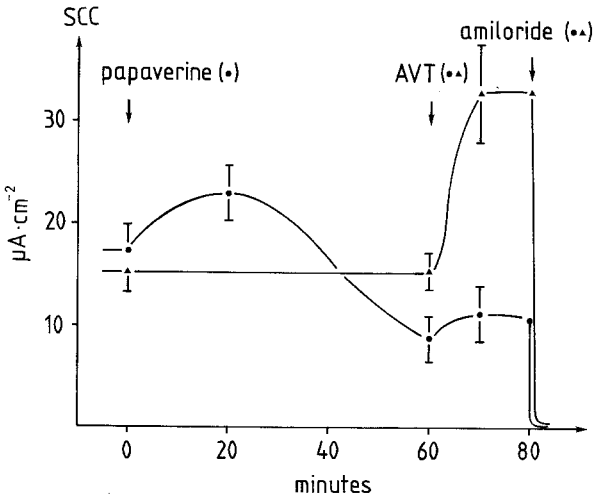

Fig. 1. The effect of papaverine (150 μM) on the SCC and the ability of AVT to activate the SCC in the presence and absence of papaverine in the basolateral solution. After 90-min incubation with papaverine in one skin half (circles) AVT (48 nM) was added to both skin halves. After reaching a maximal stimulation with AVT, amiloride (0.1 mM) was added to the apical solution of both skin halves. $n = 8$.

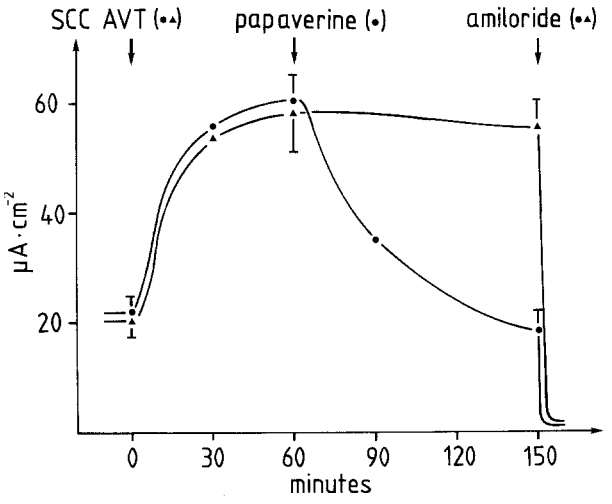

Fig. 2. The effect of papaverine (circles) on SCC in frog skins preincubated with AVT (48 nM). After maximal stimulation of the SCC with AVT (60 min), papaverine (150 μM) was added to the basolateral solution of one skin half. After 90-min incubation with papaverine, amiloride (0.1 mM) was added to the apical solution of both skin halves. $n = 6$.

tion of papaverine (Johnsen & Nielsen, 1984) and incubation time (*data not shown*).

The observed effects obtained by addition of papaverine to the basolateral solution were reversible (*see* later in Fig. 3). After four times washout of the solution with fresh Ringer's solution, the SCC returned to the level measured before the addition of papaverine; so the effects of papaverine were not due to irreversible events.

When papaverine was added to the basolateral solution of skins preincubated with AVT only an inhibition of the SCC was observed (Fig. 2).

Measurements of the transepithelial sodium fluxes showed that the effects on SCC were due to changes in the active sodium influx (Table 1).

Table 1. Comparison of net fluxes of Na^+ with SCC before and after addition of papaverine (150 μM) to the basolateral solution

Period (min)	Condition	Net flux of Na^+	
		Na^+ flux	SCC
		neg · min^{-1} · cm^{-2}	
0–30	Control	9.4 ± 3.6	10.0 ± 3.5
30–60	Control	8.8 ± 3.0	9.1 ± 3.3
60–90	Papaverine	11.4 ± 3.0	10.9 ± 3.0
90–120	Papaverine	9.3 ± 2.5	9.0 ± 1.9
120–150	Papaverine	6.0 ± 1.3	4.8 ± 1.2
150–180	Papaverine + AVT	8.7 ± 1.4	10.1 ± 2.1
180–210	Papaverine + AVT	11.1 ± 2.3	11.6 ± 2.6

After two periods under control conditions, papaverine was added to the basolateral solution. After three periods with papaverine, AVT was added to the basolateral solution (48 nM). Net influx of an ion is indicated as positive values (mean ± SE). $n = 5$.

Effect on Cellular cAMP Production

The stimulatory effect of papaverine on the SCC could be explained if papaverine as reported inhibits the phosphodiesterase responsible for breakdown of cAMP (Triner et al., 1971; Miyamoto et al., 1976). The effect of papaverine (150 μM) on the production of cAMP, both in the absence and presence of AVT, was measured in isolated epithelia (Table 2).

The concentration of cAMP under control conditions was 4.4 ± 0.8 pmol per mg dry wt. When isolated epithelia were incubated in the presence of papaverine for 20 min, the cAMP concentration doubled to 8.4 ± 1.0 pmol per mg dry wt, as expected for a component which inhibits the phosphodiesterase. After 60-min incubation with papaverine, the cAMP level was 6.7 ± 1.1 pmol per mg dry wt. The ability of AVT to stimulate the cAMP production was unaffected, even after 60-min preincubation with papaverine, at a time where a pronounced inhibition of the SCC and the natriferic action of ADH was observed. The cAMP concentration in the presence of AVT was 38.9 ± 5.3 pmol per mg dry wt, and in the presence of papaverine plus AVT the cAMP concentration was 32.5 ± 5.1 pmol per mg dry wt.

Table 2. Effect of papaverine (150 μM) and AVT (48 nM) on cellular cyclic AMP content

Conditions	cAMP (pmol/mg dry wt)[a]	n
Control	4.4 ± 0.8	8
$\text{Papaverine}_{10\,\text{min}}$	8.4 ± 1.0	5
$\text{Papaverine}_{60\,\text{min}}$	6.9 ± 1.1	5
$\text{AVT}_{10\,\text{min}}$	38.5 ± 5.3	8
$\text{Papaverine}_{60\,\text{min}}\text{AVT}_{10\,\text{min}}$	32.5 ± 5.1	8

In the experiments where the effect of AVT on the cAMP production was tested, AVT was present in the incubation medium for the last 10 min, because the production of cyclic AMP is maximal after 10-min incubation with AVT (Johnsen & Nielsen, 1984).
[a] Dry weight of the isolated epithelium.

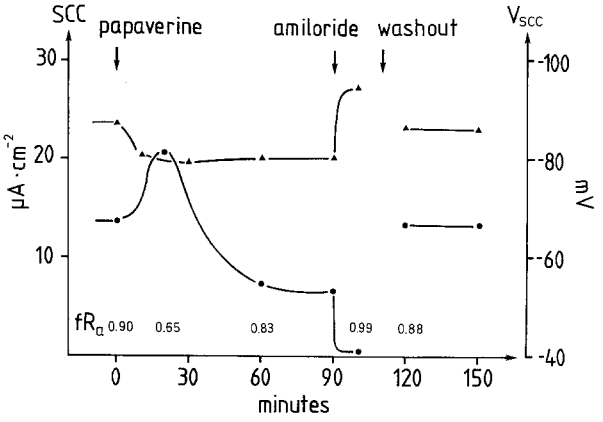

Fig. 3. Typical experiment of the time course of papaverine-induced changes in SCC (circles) and V_{scc} (triangles). Papaverine was added to the basolateral solution at time zero. After 90-min incubation with papaverine, the basolateral solution was replaced with fresh Ringer's solution, a procedure which brings the SCC and V_{scc} back to the level they had before the addition of papaverine. The fractional resistance of the apical membrane, fR_a, is noted in the figure.

Effect on Intracellular Parameters

From the responses to serosal addition of papaverine, one might suggest an effect on the sodium transport across the frog skin via action in the apical membrane. The general assumption is that, in most cases, effects on active sodium transport are connected to the changes in the sodium permeability of the apical membrane (P^a_{Na}). If so, one would expect that an increase in the current should result in a depolarization of the cell potential and a decrease in the current should result in a hyperpolarization of the cell potential.

In order to examine the effects of serosal addition of papaverine on the cellular potential under short-circuited conditions, V_{scc}, a series of impalements with microelectrodes was made.

A typical experiment is shown in Fig. 3. It is seen that addition of papaverine initially results in an activation of the SCC, in a depolarization of V_{scc} and in a decrease in the fractional resistance of the apical membrane (fR_a). These changes strongly indicate that P^a_{Na} increases.

After the initial increase the SCC started to decrease, but no effect on V_{scc} was observed.

A decrease in the SCC could be due to the following events: (i) a decrease in the potassium permeability of the basolateral membrane (P^b_K), (ii) an inhibition of the Na/K pump in the basolateral membrane, changes which would result in a depolarization of V_{scc} (Nagel, 1979; Harvey & Kernan, 1984; Nielsen, 1985), or (iii) if the cellular chloride concentration is not in equilibrium it might be due to an increase in the chloride permeability (P_{Cl}).

In order to distinguish between these possibilities we measured the cellular Na concentration, Na_c and P^a_{Na}. This was done by measuring the I/V relationship across the apical membrane both in the absence and presence of the sodium channel blocker, amiloride. The apical membrane of the isolated frog skin is sodium selective (Koefoed-Johnsen & Ussing, 1958). So the difference curve (control–amiloride) displays the sodium current across the apical membrane as a function of the transmembrane potential. This curve was fitted to the Goldman equation (Fig. 4*A* and *B*), and Na_c and P^a_{Na} were estimated from the curve. When the current is zero the potential displays the equilibrium potential for sodium and the Na_c can be calculated from the Nernst equation, and when the transmembrane potential is zero one can calculate P^a_{Na} from Ficks law.

From Table 3 it is seen that under control condition the SCC was 25.6 ± 5.5 μA cm^{-2}, P^a_{Na} was 8.5 ± 1.6 × 10^{-7} cm · sec^{-1}, Na_c was 8.6 ± 1.9 mM and V_{scc} was −82 ± 2 mV.

After 90-min incubation with papaverine the SCC was 14.2 ± 3.3 μA cm^{-2}, P^a_{Na} was 6.1 ± 2.2 × 10^{-7} cm · sec^{-1}, Na_c was 4.3 ± 1.3 mM and V_{scc} was −74 ± 3 mV.

Thus 90-min incubation with papaverine resulted in a 45%. reduction in the SCC, a 28% decrease in P^a_{Na}, a 50% reduction in Na_c and a 10% depolarization of V_{scc}.

So the observed inhibitory effects of papaverine on SCC was partially due to a reduction in P^a_{Na}, but it was also associated with a decline in V_{scc}, indicating that papaverine also had an effect on parameters other than the sodium permeability. This could be due to a decrease in the cellular potassium permeability and/or enhanced cellular chloride permeability, but it could not be due to an inhibitory effect of papaverine on the Na/K pump, because this should

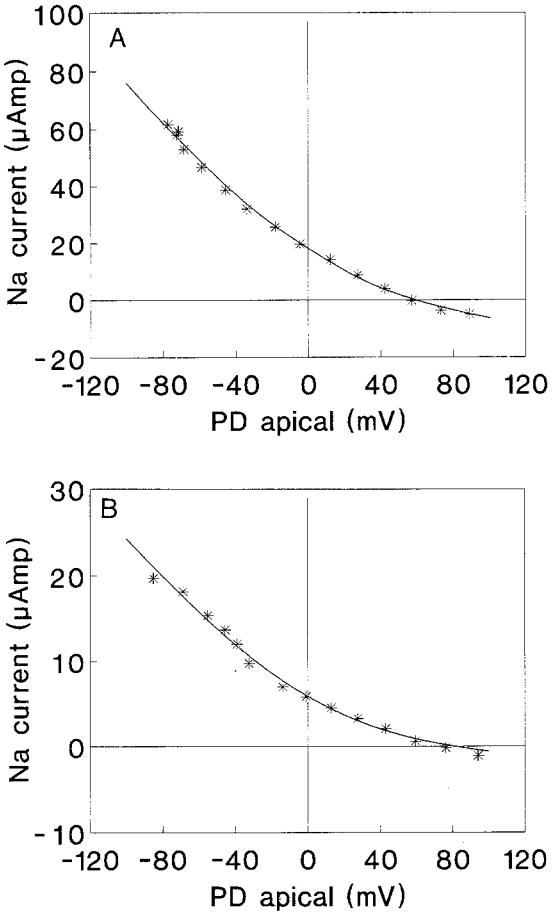

Fig. 4. Typical experiment of the steady-state current-voltage relationship of the apical membrane in the frog skin under control condition (*A*) and in frog skin incubated with papaverine for 90 min (*B*). The data displays the difference values obtained when subtracting the amiloride data from the control data (for further information, *see* text) and are fitted to the Goldman equation (solid line) as described in the text.

result in an increase in the cellular sodium concentration and not a decrease. As seen in Table 3, the presence of ouabain for 30 min resulted in an increase in the Na_c from 8.2 to 12.9 mM ($n = 2$).

To examine whether the effects of papaverine were a result of enhanced cellular chloride permeability, a series of experiments were carried out under conditions where chloride was in equilibrium. This was done by blocking the Na,K,2Cl cotransporter with the addition of 50 μM bumetanide to the serosal solution. The skin was preincubated with bumetanide for 60 min before the addition of papaverine. From Fig. 5 it is seen that 60-min incubation with bumetanide had no effect on either the SCC or the V_{scc}. In the presence of bumetanide, the addition of papaverine resulted as shown previously (Fig. 1) in a transient increase in the SCC followed by a decrease in the SCC (Fig. 5). The V_{scc} was -82 ± 2 mV and started to depolarize as the SCC began to increase. After 30-min incubation with papaverine the cells were depolarized to -52 ± 3 mV. Then the cells hyperpolarized, and after 90-min incubation with papaverine (150 min with bumetanide), a new steady-state level of the V_{scc} was -79 ± 2 mV. After a transient increase of the SCC, the incubation with papaverine caused the SCC to decrease from 17.0 ± 1.2 $\mu A\ cm^{-2}$ to 8.1 ± 1.1 $\mu A\ cm^{-2}$ and a net depolarization of the V_{scc} from -82 mV to -79 mV.

The effect of bumetanide on skins preincubated with papaverine was also investigated. From Fig. 6 it is seen that 90-min incubation with papaverine resulted in the transient increase in the SCC followed by a decrease in the SCC. The basal level was 12.1 ± 1.2 $\mu A\ cm^{-2}$, and after 90 min, the SCC was 8.7 ± 0.8 $\mu A\ cm^{-2}$ and V_{scc} depolarized from -84 ± 3 mV to -77 ± 3 mV. Bumetanide was then added to the serosal solution. After 60-min incubation with bumetanide (150-min with papaverine), the SCC was 6.9 ± 0.8 $\mu A\ cm^{-2}$ and V_{scc} was 79 ± 3 mV. Since bumetanide had no effect on V_{scc}, either in the presence or in the absence of papaverine, the effect of papaverine on the V_{scc} could not be explained by an increase in the cellular chloride permeability. The observed depolarization of V_{scc} as SCC decreases in the presence of papaverine could be understood if papaverine decreases P_K^b in the frog skin epithelium.

Discussion

From the data presented it is seen that serosal addition of papaverine causes a biphasic response in the SCC of the isolated frog skin; an initial increase in the SCC is followed by a pronounced decrease in it (Fig. 1). When papaverine was added to skins where the Na transport had been activated previously by addition of AVT only the inhibitory effect of papaverine was observed (Fig. 2). Both the stimulatory and inhibitory effects of papaverine were reversible (Fig. 3), which therefore seems to be mediated via specific reactions in the processes responsible for the Na transport across the frog skin.

The initial activation of the Na transport after addition of papaverine is probably due to the fact that papaverine is a phosphodiesterase inhibitor (Triner et al., 1971; Lugnier & Stocklet, 1974; Miyamoto et al., 1976). This notion is supported by the fact that papaverine does not activate the current across skins where the current had been activated previous by AVT (Fig. 2). Furthermore, the addition of papaverine *per se* doubled the cellular concentration of cAMP (Table 2) in agreement with findings by Poch and Kukovetz (1971).

Table 3. Effect of papaverine (150 μM) after 90-min incubation in the basolateral solution of P^a_{Na}, Na_c, V_{scc} and the SCC compared to control halves[a]

	SCC ($\mu A/cm^{-2}$)	P^a_{Na} (10^{-7} cm/sec)	Na_c (mM)	V_{scc} (mV)	n
Control	25.6 ± 5.5	8.5 ± 1.6[b]	8.6 ± 1.9	−82 ± 3	6
Papaverine	14.2 ± 3.3	6.1 ± 2.2[b]	4.3 ± 1.3	−74 ± 3	6
Control	25.5	—	8.2	−84	2
Ouabain	8.0	—	12.9	−49	2

[a] n is noted in the table. Two experiments where the skins have been incubated with ouabain (0.1 mM) for 30 min are included in the table.
[b] $0.05 < P < 0.01$ (paired t test).

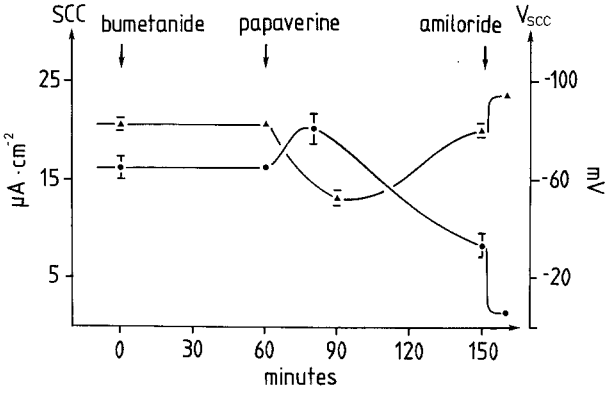

Fig. 5. Time course of papaverine-induced changes in SCC (circles) and V_{scc} (triangles) when added to skins preincubated with bumetanide. Bumetanide (50 μM) was added to the basolateral solution at time zero. After 60-min incubation with bumetanide, papaverine (150 μM) was added to the basolateral solution. After 90-min incubation with papaverine (150 min with bumetanide), amiloride (0.1 mM) was added to the apical solution. n = 6.

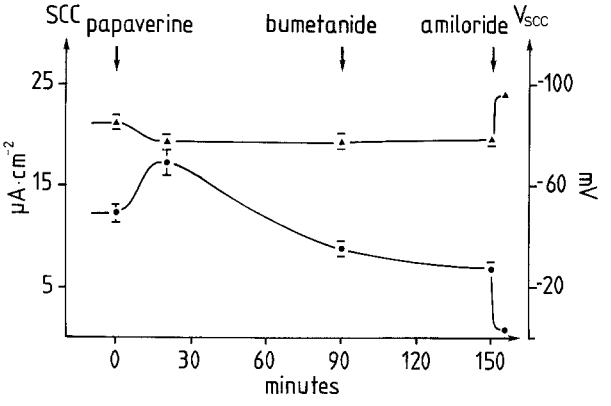

Fig. 6. Time course of bumetanide (50 μM) induced changes in SCC (circles) and V_{scc} (triangles) when added to skins preincubated with papaverine for 90 min. Papaverine (150 μM) was added to the basolateral solution at time zero. After 90-min incubation with papaverine, bumetanide was added to the basolateral solution. After 60-min incubation with bumetanide (150 min with papaverine), amiloride (0.1 mM) was added to the apical solution. n = 5.

It is well known that inhibition of the cellular breakdown of cAMP results in an activation of the transepithelial Na transport (Baba, Smith & Townshend, 1967; Rajerison et al., 1972; Johnsen & Nielsen, 1984). Measuring the transepithelial net flux of sodium by isotope tracer (Na^{22}) revealed that the effects on SCC were in fact due to transepithelial Na transport.

The transepithelial Na transport across isolated frog skin can be described by the model presented in Fig. 7. For references *see* Kristensen and Ussing (1985) and Larsen (1988, 1991). According to this model, the cellular potential under short-circuited conditions (V_{scc}) can be described by the Goldman-Hodgkin-Katz equation (GHK):

$$V_{scc} = \frac{RT}{zF} \ln \frac{P_K K^o + P_{Na} Na^o + P_{Cl} Cl^c}{P_K K^c + P_{Na} Na^c + P_{Cl} Cl^o} \qquad (2)$$

where R, T, z and F have their usual meanings, P is the cellular permeability of the ion noted as subscript, and the superscripts o and c refers to outside and cellular ion concentration, respectively. The electrogenic contribution, which normally is about 5 mV (Nagel, 1980), has been omitted from the GHK equation.

The model predicts that an inhibition of the SCC might be due to an inhibition of the Na/K pump or a decrease in P^a_{Na} or P^b_K or in an increase in P_{Cl} (*see below*). A decrease in P^a_{Na} would result in a hyperpolarization of V_{scc}, as observed when the sodium channels are blocked with amiloride (Fig. 3), in agreement with findings described by Larsen (1973) and Harvey and Kernan (1984). A decrease in P^b_K would result in a depolarization of V_{scc} and an inhibition of the transepithelial Na transport (Nagel, 1979; Nielsen, 1985). Inhibition of the Na/K pump would also result in a decrease in the current and a depolar-

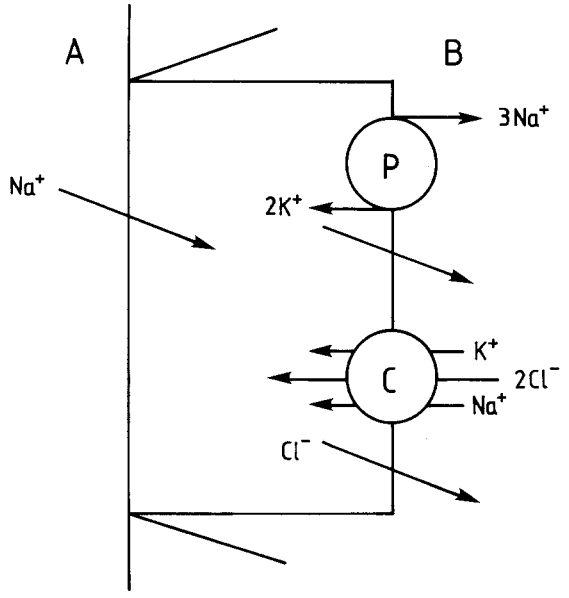

Fig. 7. Transport model depicting major pathways responsible for uptake of sodium in principal cells in the frog skin epithelium. Downhill arrows are passive pathways. *P*, primary active transport systems. *C*, cotransport system. *A* and *B* refer to apical and basolateral membrane, respectively.

ization of V_{scc}. The depolarization is partially due to a decrease in the electrogenic contribution from the Na/K pump and partially due to the fact that the cellular Na concentration increases and the cellular K concentration decreases (Harvey & Kernan, 1984). Finally, an increase in P_{Cl} might also result in a depolarization of V_{scc} if the chloride equilibrium potential were more positive than the membrane potential before any possible P_{Cl} increase (Ferreira & Ferreira, 1981; Larsen, 1991).

As papaverine stimulates the SCC we observed a depolarization of the V_{scc}, as expected for a component which stimulates the active Na transport by increasing P^a_{Na}.

After the initial activation the presence of papaverine resulted in a decline in the SCC. Since papaverine stimulated the production of cAMP (Table 2), this inhibition had to interfere with a step distal to the activation of the adenylate cyclase. A possible target could be an inhibition of P^a_{Na}, but in this case one would expect the cells to hyperpolarize.

From the data presented in Table 3 it is seen that 90-min incubation with papaverine resulted in a 45% reduction in the SCC, a 28% decrease in P^a_{Na}, a 10% depolarization of V_{scc} and a 50% reduction of the cellular Na concentration. Thus the inhibitory effect of papaverine was associated with a depolarization of V_{scc} and not a hyperpolarization; this indicates that papaverine had an effect on parameters other than P^a_{Na}. The depolarization was not due to an inhibition of the Na/K pump because the inhibition of the SCC was associated with a decrease in the cellular Na concentration (Table 3). It is known that an inhibition of the Na/K pump by addition of ouabain results in a depolarization of V_{scc} together with a decrease in P^a_{Na} (Erlij & Smith, 1973). Harvey and Kernan (1984) showed that addition of ouabain inhibited the Na transport and increased Na_c. They found that P^a_{Na} was unchanged until the Na_c level was above 15 mM. So the inhibitory effect of papaverine on the Na transport is located to the sodium channels in the apical membrane.

The depolarization was not due to an inhibition of the Na/K pump, so papaverine must also have an effect on P^b_K or P_{Cl}. When eliminating the apical Na permeability by adding amiloride to the mucosal solution, the contribution of sodium in the GHK equation can be omitted, and the only contributions are K and Cl. The fact that amiloride hyperpolarizes the cells to above -100 mV (close to the equilibrium potential for K) indicates that P_{Cl} must be very low compared to P^b_K.

In experiments where the basolateral Na,K,2Cl cotransporter was blocked with the loop-diuretic bumetanide (so the cellular chloride concentration can be assumed to be in equilibrium), papaverine elicited its usual effect on the SCC and V_{scc}. This means that the contribution of chloride to the cellular potential is negligible. Consequently the observed changes cannot be due to changes in P_{Cl}. It is therefore suggested that the observed depolarization is due to a decrease in P^b_K.

Thus the data presented indicate that the observed inhibitory effect of papaverine is due to a decrease in P^a_{Na} and P^b_K, and since the cells depolarize, the decrease in P^b_K has to be relatively greater than the decrease in P^a_{Na}.

The effect of papaverine could be mediated via a calcium-sensitive pathway. Papaverine has been demonstrated to act as a calcium antagonist in tracheal smooth muscle (Ito & Itoh, 1984), in uterus (Villar, D'Ocon & Anselmi, 1986) and in portal vein (Dacquet, Mironneau & Mironneau, 1987). According to these observations, the effect of papaverine is not related to external calcium, since removal of calcium did not abolish the induced contractions of the muscle cells. The effects were related to the release of calcium from intracellular stores, which seemed to be the same for different agonists (Villar et al., 1986). If papaverine also acts as a calcium channel blocker in isolated frog skin, then the incubation with papaverine might lead to a decrease in the cellular calcium activity.

When frog skins were incubated in calcium-free

Ringer's solution it resulted in an increase in the affinity of the skin for AVT (the amount of AVT necessary to obtain half-maximal activation of the current) (Johnsen & Nielsen, 1982). In the presence of papaverine the affinity for AVT also increased (H. Andersen & R. Nielsen, *unpublished*). These data are in agreement with the notion that incubation with papaverine leads to a decrease in the cellular calcium activity. A decrease in the cellular calcium activity is known to result in a decrease in the cellular prostaglandin synthesis (Els & Helman, 1981, Erlij, Gersten & Sterba, 1981; Nielsen, 1984). A decrease in the cellular content of prostaglandin E_2 (PGE_2) would result in a decrease in P^a_{Na} (Helman, Cox & van Driessche, 1983), as we have observed in the present experiments.

In different tissues, such as choroid plexus (Christensen & Zeuthen, 1987), thick ascending limp of Henle's loop (Klaerke & Jørgensen, 1988) and rabbit distal colon (Loo and Kaunitz, 1989), it has been shown that the K channel responsible for the transmembrane K movement is activated by calcium. If the K channels in the isolated frog skin also were calcium activated, then one might expect that a decrease in the cellular calcium acitivity might lead to a decrease in P^b_K, as observed in the present experiments.

In a recent abstract (Harvey et al., 1991) it has been shown that the basolateral membrane of the isolated frog skin contains both K channels which are activated by calcium and K channels which are inhibited by calcium, and it is claimed that it is the calcium-inhibited K channels which are responsible for the K movement across the basolateral membrane. Thus according to these observations a decrease in the cellular calcium activity should lead to an increase in P^b_K.

In experiments where V_{scc} was measured in frog skins bathed in calcium-free medium, it was found that this resulted in a slight depolarization which is in agreement with the assumption that a decrease in cellular calcium activity should lead to a decrease in P^b_K (Andersen & Nielsen, 1989).

Thus at the present stage, the data concerning the effect of calcium on P^b_K are conflicting.

In conclusion, the data presented shows that serosal addition of the opiate derivative papaverine results in a doubling of the cellular content of cAMP and an increase in the transepithelial Na transport. But prolonged incubation with papaverine resulted in a pronounced decrease in the transepithelial Na transport and natriferic action of AVT. The effect of papaverine was reversible. The decrease in Na transport was due to a simultaneous decrease in the apical sodium permeability and the basolateral potassium permeability. The inhibitory effect of papaverine could be mediated via changes in the cellular calcium.

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