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EFFECT OF HARMALINE ON SODIUM TRANSPORT IN *Rana esculenta* SKIN

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1 Harmaline, together with certain hallucinogenic alkaloids of the same group (harmine, 2 methyl harmine) stimulates sodium transport across the *in vitro* skin of *Rana esculenta* when it is added to the external medium at a low concentration (0.1 mM). This effect is due to an increase of the sodium influx, and is reversed by washing. It is suggested that harmaline intervenes at the sodium penetration sites at the external face of the transport compartment.

2 At a higher concentration (5 mM) added to the internal medium harmaline inhibits sodium net absorption. The inhibition is due mainly to an increase of the efflux, while the influx may be either inhibited or increased. Under these conditions the influx becomes insensitive to amiloride. It is suggested that the inhibition of sodium transport is the result of harmaline interfering with a transport ATPase, and also that harmaline induces new sites for the passage of sodium.

Introduction

Harmaline, like certain compounds of the indole-alkaloid group is a hallucinogen (Hoffer & Osmond, 1967; Schultes, 1969). According to Canessa, Jaimovich & de la Fuente (1973) these substances act as Na/K ATPase inhibitors at the Na^+-dependent phosphorylation level. They noted an inhibitory effect of harmaline in ATPase preparations of human red blood cells, rat brain and squid retinal axon and also showed that harmaline reduced sodium transport across the squid axon and in frog skin. Sepulveda & Robinson (1974) reported inhibition of sodium transport in mammalian intestinal mucosa and kidney cells by harmaline and suggested that harmaline interacts with the sodium binding sites of the transport carriers.

The experiments on the isolated skin of *Rana esculenta* recorded here, show that harmaline modifies the sodium transport of this epithelium. Added to the epithelial face of the preparation it stimulates sodium transport while adition to the serosal face results in a diminution of the short circuit current. It is proposed that the activating effect of harmaline is an action on the permeability of most external barriers while the inhibitory effect is probably due to interference between harmaline and a transport ATPase.

Preliminary accounts of some of these observations have been published (Ehrenfeld & Garcia-Romeu, 1975).

Methods

The experiments were carried out on isolated abdominal skins of *Rana esculenta*. The animals were kept, unfed, in running tap water at 15°C. The skins were mounted in lucite double chambers, the exposed areas being 7 cm^2 or 1.54 cm^2 according to the type of chamber used. Each side of the skin was bathed with either 7 ml or 3 ml. of Ringer, circulated by an air-stream. The potential difference (PD) between epithelial and corial bathing solutions was measured with calomel electrodes and agar-KCl bridges. For measurement of the short circuit current (SCC) the counter potential was applied with Ag–AgCl electrodes coupled to an automatic SCC apparatus. All experiments were preceded by an equilibration period of an hour.

For the measurements of Na^+ influx, ^{22}Na (20 μCi/100 ml) was added to the epithelial bathing solution and after 20 min for equilibration, its appearance in the internal chamber was followed with time. The radioactivity of the samples was measured in a Mecaserto well counter MO 13/100. Influxes were calculated from the quantity of radioisotope transferred per unit time and the specific radioactivity in the external compartment.

When the mucous face of the skin was bathed with dilute NaCl solution (2 mM) the sodium transport was studied in open circuit conditions and sodium concentration was measured by flame photometry with an

Eppendorf photometer. The net fluxes of Na^+ were calculated from the slopes of the sodium concentrations in the external medium as a function of time. In the 2 mM/Ringer experiments the sodium efflux was obtained as the difference between influx, measured with ^{22}Na, and netflux. All fluxes are expressed in nEq h^{-1} cm^{-2}.

Ringer solution of the following composition (mM) was used; NaCl 111, $NaHCO_3$ 2.4, KCl 2.0, $CaCl_2$ 1.0 and glucose 11.1.

Dilute sodium chloride medium contained 2 mM NaCl and 2 mM imidazole adjusted to pH 7.2 with H_2SO_4; amiloride (Merck, Sharp & Dohme Research Laboratories, West Point, Pa) was added to the external medium; ouabain (Calbiochem) was added to the internal medium; harmaline, harmine and 2 methyl harmine (Sigma Chemical Co., St. Louis, Missouri) were added either to the external or internal medium.

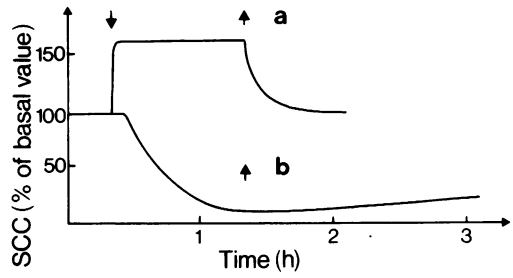

Figure 1 Effect of harmaline on short circuit current (SCC) added to the external (a) or internal (b) medium. Concentrations: external medium 0.2 mM, internal 5 mM. Addition of hallucinogen and washing marked by arrows. Ordinate scale: variation of SCC as per cent of basal value; abscissa scale: time in hours. The values of SCC in experiment (a) were: control 1803 nEq h^{-1} cm^{-2}, in the presence of harmaline 2884 nEq h^{-1} cm^{-2} and after washing 1790 nEq h^{-1} cm^{-2}. In experiment (b) control 1205 nEq h^{-1} cm^{-2}, in the presence of harmaline (maximal effect) 125 nEq h^{-1} cm^{-2} and after washing 241 nEq h^{-1} cm^{-2}.

Results

Stimulation by harmaline added to the external medium of transepithelial sodium transport

Harmaline (0.2 mM) added to the epithelial face of the skin produced an immediate increase of the SCC (Figure 1a) and of the transepithelial PD and also a slight reduction of the resistance (Table 1). Table 2 A shows that this SCC increase is due to an increase of the sodium influx. The SCC increase was immediate but the time for the maximal response was variable: frequently it was approximately 1 min but varied from 10–20 s to 20 minutes. In the continued presence of harmaline the SCC values were relatively stable with a tendency to fall with time. The SCC and the PD returned to their normal values when preparations were washed.

When added to the external medium at a concentration of 0.2 mM harmine and 2 methyl harmine have effects similar to those of harmaline on the SCC; the stimulation of the SCC induced by these three chemically related compounds was respectively 57%, 60% and 44%. The stimulatory effect was in all instances reversible.

When the skin was mounted in open circuit with the epithelial face bathed with dilute NaCl, addition of harmaline (0.1 mM) to this solution increased the sodium netflux due to an augmentation of the influx (Table 2B).

Concentration-response curve: harmaline added to external medium

Figure 2 shows that the threshold of the stimulatory effect of harmaline was between 1 and 10 μM and its maximal effect was at 1 mM. Higher concentrations

Table 1 Variation of the electrical parameters of the frog skin after addition of harmaline

	PD	*SCC*	*R*
Control ($n=7$)	86.3 ± 4.6	1790 ± 153	1879 ± 211
Harmaline 0.2 mM ($n=7$)	110.0 ± 6.4	2631 ± 201	1625 ± 185
Diff.	+23.7 ± 4.5**	+841 ± 130**	−254 ± 95*

Short circuit current (SCC), potential difference (PD) and transepithelial resistance (R) in nEq h^{-1} cm^{-2}, mV and Ω/cm^2 respectively before and after the addition of harmaline (0.2 mM) to the external medium. Number of skins in parentheses. Diff.: Difference of paired data ± s.e. mean.
* $P<0.05$; ** $P<0.001$.

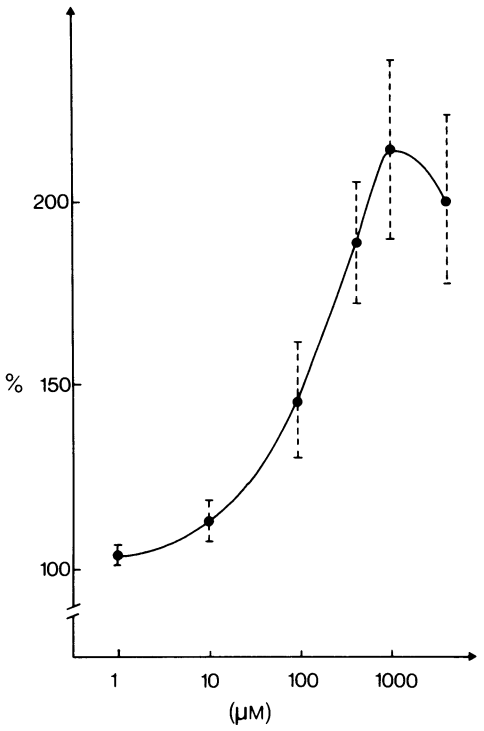

Figure 2 Concentration-response curve to harmaline added to external face of skins. Ordinate scale: short circuit current (SCC) as per cent increase relative to basal value; abscissa scale: logarithm of hallucinogen concentration. Number of experiments $n=5$. Mean value of initial current 728 ± 177.

(5 mM) stimulated the SCC less than 1 mM. At these concentrations current stimulation was sometimes followed by a slight inhibition, giving a biphasic response.

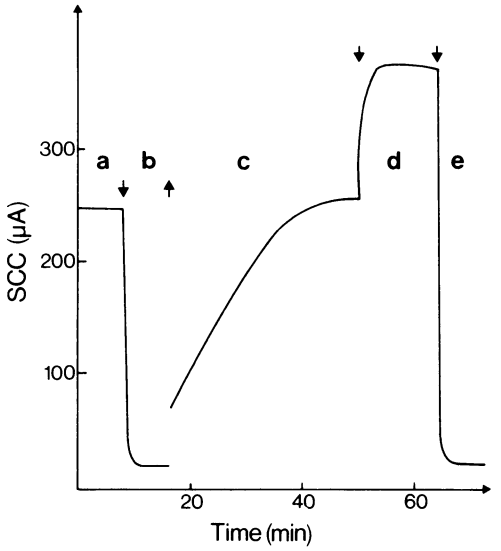

Figure 3 Short circuit current (SCC) of a skin during 5 successive periods; (a) control, (b) addition of amiloride (10 μM) to external medium, (c) washing, (d) addition of harmaline (0.2 mM) to the external medium, (e) addition of amiloride (10 μM) in presence of harmaline. Ordinate scale: SCC in μA; abscissa scale: time in minutes. Area 7 cm^2.

Amiloride-harmaline interactions

To investigate interactions between amiloride (an inhibitor of Na ion translocation at the external face) and harmaline, inhibition by amiloride was studied in the presence and absence of harmaline. Amiloride was used at a concentration of 10 μM and harmaline added to the external medium at a concentration of 0.2 mM. One such experiment is shown in Figure 3. After a control period (a) amiloride added in the external

Table 2 The effect of harmaline applied to the external solution on transepithelial sodium transport

		J_{in} Na^+	SCC	
	Control	1008 ± 184	1080 ± 224	
A	Harmaline	1222 ± 194	1395 ± 198	
($n=8$)	Diff.	214 ± 55***	315 ± 97**	
		J_{in} Na^+	J_{net} Na^+	J_{out} Na^+
	Control	140 ± 27	+71 ± 26	69 ± 19
B	Harmaline	193 ± 33	+105 ± 32	88 ± 24
($n=5$)	Diff.	53 ± 10***	34 ± 10*	19 ± 7*

Two experimental conditions: (A) short circuited skins bathed in Ringer solutions, (B) open-circuit skins with external face bathed with dilute (2 mM) NaCl solution. J_{in}, J_{out}, SCC, J_{net}=influx, efflux, short circuit current and net flux respectively. All values in nEq h^{-1} cm^{-2} ± s.e. mean. In expts A harmaline added in concentration of 0.2 mM; in B 0.1 mM. Number of skins in parentheses. Diff: Difference of paired data ± s.e. mean.
*$P<0.05$; **$P<0.02$; ***$P<0.01$.

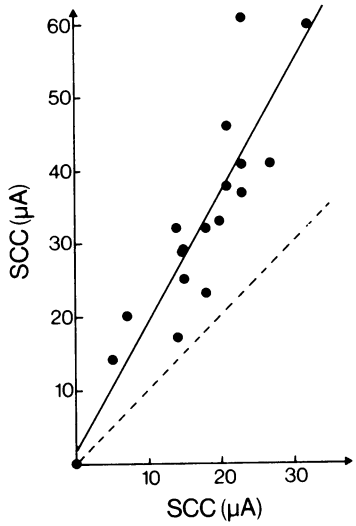

Figure 4 Stimulation of short circuit current (SCC) by harmaline (0.1 mM) added to external medium after various degrees of inhibition of Na transport by ouabain. Ordinate scale: maximal response of SCC after addition of harmaline to external medium; abscissa scale: SCC remaining after ouabain treatment. SCC expressed in μA. Area: 7 cm^2. Equation: SCC (ouabain + harmaline) = 1.77 SCC (ouabain) + 1.36 (r = 0.93).

medium induced a strong inhibition of sodium transport (b) and washing restored the SCC to a value similar to the control period (c). Addition of harmaline induced a stimulation of the SCC (d) and amiloride (e) in the presence of harmaline inhibited the sodium transport to the same extent as in period (b). The means and standard errors of transport rates (in nEq h^{-1} cm^{-2}) on a group of 4 skins were as follows: control 1535 ± 273; amiloride in the external medium 183 ± 48; washing 1500 ± 371; addition of harmaline to the external medium 2031 ± 448; addition of amiloride in presence of harmaline 170 ± 38. The current level after amiloride inhibition was the same irrespective of the presence of harmaline. Furthermore the addition of harmaline (0.2 mM) in the presence of amiloride (1 μM) had no effect on SCC.

The effect of external harmaline on ouabain-pretreated skins

The stimulatory effects of harmaline (0.1 mM) were studied on skins of *R. esculenta* previously treated with ouabain in concentrations varying from 5 μM to 5 mM. The results from 4 groups of 5 animals are represented in Figure 4. The increase of SCC brought about by the addition of harmaline was found to be proportional to the SCC value remaining after inhibition by ouabain. When the inhibition was total, harmaline could not restore Na transport.

Action of harmaline added to the internal medium

Table 3 summarizes the effect on the SCC of the addition of harmaline at two different concentrations to the internal medium. At 0.1 mM, after a long latent period, it produced a slight transitory increase of the SCC with a maximum at about 20 minutes. At a dose 50 times higher (5 mM) it reduced the SCC (Figure 1b, Table 3); this inhibition started after a few minutes, was about 30% after 30 min and 85% after 1.5 hours. It was not readily reversed by washing.

In a different type of experiment, unidirectional Na^+ fluxes and the SCC were followed after the addition of harmaline to the internal medium (Table 4). The experiments covered 3 periods of 45 min: a control period, one in which harmaline (5 mM) was added to the internal solution and one with both external amiloride (50 μM) and harmaline in the internal medium. Following the control period, measurements were started 90 min after the addition of the harmaline. The results of 25 skins tested in this way

Table 3 The effect of harmaline (5 mM or 0.1 mM) added to the internal medium

Dose	*Control t=0*	*t=30 min*	*t=60 min*	*t=90 min*
5 mM	1085 ± 182 (n=10)	759 ± 165 (n=10)	384 ± 85 (n=10)	165 ± 87 (n=9)
% inhibition		30%	65%	85%
	t=0	*t=20 min*	*t=44 min*	
0.1 mM	812 ± 125 (n=8)	919 ± 141 (n=8)	788 ± 146 (n=8)	

After a control period, harmaline was added to the internal medium and the SCC followed as a function of time. Current in nEq h^{-1} cm^{-2}. Number of skins in parentheses. Decrease of the SCC in per cent.

fell into two groups: group A in which harmaline reduced the sodium influx and group B in which it did not. In both groups the addition of harmaline to the internal medium made the epithelium incapable of carrying out a net sodium transport since the effluxes were increased to the same level as the influxes. In these conditions the influx of Na was no longer sensitive to amiloride.

Discussion

Harmaline added at 10 μM to 0.5 mM to the mucosal face of the skin increases the transepithelial transport of sodium. This stimulation of Na^+ net transport is due to an increase of Na^+ influx and was found to occur under two experimental conditions: when the skin is short-circuited and bathed on both sides by Ringer, and when it is in open circuit conditions with a dilute sodium chloride solution at the external face. An accepted model of the penetration of sodium is that there are two barriers limiting the transport compartment: an external one permeable to sodium and impermeable to potassium (Koefoed-Johnsen & Ussing, 1958) which sodium penetrates by facilitated diffusion (Cereijido, Herrera, Flanigan & Curran, 1964; Biber & Curran, 1970; Biber & Sanders, 1973; Erlij & Smith, 1973) and one represented by the basal and lateral membranes which are impermeable to sodium but permeable to potassium (Koefoed-Johnsen & Ussing, 1958; Ussing, 1960; Ussing & Winhager, 1964; Farquhar & Palade, 1965). On these membranes an ATPase controlling Na^+ absorption to the internal medium is thought to be localized (Farquhar & Palade, 1965). At which of these levels does harmaline exert its effect? We consider it unlikely that it acts directly on the sodium pump by stimulating an ATPase. Canessa *et al.* (1973) have shown that harmaline (5 mM) inhibits the (Na-K) ATPase activities of various membranes. On the other hand there are some indications that its activating action is due to a reaction at the external barrier of the sodium transport compartment. Thus: (1) sodium transport only increases when harmaline is added to the external face of the preparation, (2) the SCC response to addition of harmaline is extremely rapid, and the reversal after washing is also rapid.

Amiloride reduces sodium transport by blocking the entrance of Na^+ into the transport compartment (Biber, 1971). In the present experiments amiloride completely stopped the stimulatory effect of harmaline. This suggests that the additional current recorded in the presence of harmaline passes by the same 'blockable channels' as that present in control conditions.

Ouabain inhibits the Na^+ transport by acting mainly on the active pump. When this is totally inhibited by ouabain (SCC = 0) experiments showed that the active transepithelial flux can no longer be stimulated by harmaline. If the SCC is partially reduced by ouabain, harmaline stimulates Na^+ net transport in proportion to the SCC. These experiments support the suggestion (Rick, Dörge & Nagel, 1975) that the sodium pump is not the factor limiting transepithelial Na^+ transport.

The stimulatory effects on sodium transport of the indole alkaloids used in the above experiments can be compared with the effects of certain imidazolines such as 2 guanidinbenzimidazole (GBI) or phentolamine. Thus, Zeiske & Lindeman (1974) and Garcia-Romeu

Table 4 Unidirectional sodium fluxes before and after addition of harmaline (5 mM) to the internal solution

			Control	*Harmaline (internal) (5 mM)*	*Harmaline (internal) and amiloride (external, 50 μM)*
A	($n=13$)	J_{in}	2054 ± 350	585 ± 186	
		SCC	1946 ± 324	256 ± 82	
		J_{out}	108	329	
	($n=6$)	J_{in}	2798 ± 534	897 ± 257	1326 ± 606
		SCC	2546 ± 490	331 ± 169	62 ± 37
		J_{out}	252	566	1264
B	($n=12$)	J_{in}	1666 ± 214	2518 ± 342	
		SCC	1556 ± 191	228 ± 126	
		J_{out}	110	2290	
	($n=7$)	J_{in}	1525 ± 328	2487 ± 613	2499 ± 670
		SCC	1443 ± 295	305 ± 190	74 ± 75
		J_{out}	82	2182	2425

In a third period the fluxes were measured in the presence of harmaline as before but with the addition of amiloride (50 μM) to the external medium. Harmaline period started 90 min after addition of alkaloid. The 25 skins tested fall into 2 groups: (A) harmaline reduces Na influx, (B) does not. All fluxes in nEq h^{-1} cm^{-2}. Number of animals in parentheses.

(1974) have shown that GBI in the external medium increases sodium transport by affecting the permeability of the external barrier of the transport compartment. The curves of SCC changes, their times of response and reversibility after GBI treatment are all comparable with those after harmaline treatment.

The *in vivo* crayfish gill reacts differently from the frog skin to harmaline in the external medium. In fact, the Na^+ transport of the gill is inhibited by the hallucinogen (Ehrenfeld & Garcia-Romeu, 1975) as it also is by GBI (unpublished results). As the effective dose is low (47% inhibition of influx with 5 μM harmaline and 80% with 0.2 mM) and the effect reversible, it is probable that the inhibitory action of harmaline in the crayfish is a result of interaction with sodium transport sites at the external face and not by way of an ATPase (Ehrenfeld & Garcia-Romeu, 1975). These substances, which possess nitrogen-containing heterocyclic nuclei, may be assumed to interfere with the passage of sodium at the external barrier. Their inhibitory or stimulatory actions are probably related to the nature of the apical membrane and unconnected with any action on a transport ATPase. This interpretation is in agreement with the conclusions of Sepulveda & Robinson (1974).

The addition of harmaline to the internal medium at a low concentration (0.1 mM) has little effect on the SCC. At higher concentrations (5 mM) the SCC falls progressively, and washing only slowly reverses this effect. This SCC diminution was noted by Canessa *et al.* (1973) with harmaline concentrations comparable with those used here (1 to 5 mM). Although under normal conditions the SCC represents the Na^+ net flux, under the influence of a drug this need not necessarily be the case. An SCC of zero may result from a variation of either of the unidirectional fluxes. It is essential to know these fluxes in order to interpret the results. Table 4 shows that addition of harmaline to the internal medium gave variable values for influx although all skins were treated in the same way. The results were therefore separated into two groups according to whether harmaline inhibited (Group A) or not (Group B) the sodium influx. In neither group did the epithelium produce a sodium net flux: the effluxes increased and became equal to the influxes and the addition of amiloride (50 μM) did not inhibit the sodium influx. Canessa *et al.* (1973) showed on different preparations that harmaline inhibits (Na-K) ATPases at concentrations higher than 1 mM. The inability of the epithelium to maintain a sodium net flux could be interpreted as being due to an interference of harmaline with a transport ATPase. The persistence of unidirectional influxes after harmaline treatment in one group of skins is not inconsistent with the possibility of the Na pump being inhibited. The increase of the sodium efflux and the presence of an influx insensitive to amiloride suggest that harmaline induces sites for sodium transfer other than those taken by the ion under normal conditions. It is possible that the hallucinogen increases intercellular sodium movements, perhaps by increasing the permeability of the tight junctions.

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References

BIBER, T.U.L. (1971). Effect of changes in transepithelial transport on the uptake of sodium across the outer surface of the frog skin. *J. gen. Physiol.*, **58**, 131–144.

BIBER, T.U.L. & CURRAN, P.F. (1970). Direct measurements of uptake of sodium at the outer surface of the frog skin. *J. gen. Physiol.*, **56**, 83–99.

BIBER, T.U.L. & SANDERS, M.L. (1973). Influence of transepithelial potential difference on the sodium uptake at the outer surface of the isolated frog skin. *J. gen. Physiol.*, **61**, 529–551.

CANESSA, M., JAIMOVICH, E. & DE LA FUENTE, M. (1973). Harmaline: Competitive inhibitor of sodium ion in the $(Na^+ + K^+)$-ATPase system. *J. Membrane Biol.*, **13**, 263–282.

CEREIJIDO, M., HERRERA, F.C., FLANIGAN, W.J. & CURRAN, P.F. (1964). The influence of sodium concentration on sodium transport across frog skin. *J. gen. Physiol.*, **47**, 879–893.

EHRENFELD, J. & GARCIA-ROMEU, F. (1975). Action de la harmaline sur le transport de sodium à travers la peau isolée de *Rana esculenta* et la branchie d'*Astacus leptodactylus*. *C.R. Acad. Sci. Paris*, **281**, 547–550.

ERLIJ, D. & SMITH, M.W. (1973). Sodium uptake by frog skin and its modification by inhibitors of transepithelial sodium transport. *J. Physiol., Lond.*, **228**, 221–239.

FARQUHAR, M.G. & PALADE, G.E. (1965). Adenosine triphosphatase in amphibian epidermis. *J. cell Biol.*, **30**, 359–379.

GARCIA-ROMEU, F. (1974). Stimulation of sodium transport in frog skin by 2-Imidazolines (Guanidinbenzimidazole and Phentolamine). *Life Sciences*, **15**, 539–542.

HOFFER, A. & OSMOND, H. (1967). The Hallucinogens. New York: Academic Press.

KOEFOED-JOHNSEN, V. & USSING, H.H. (1958). The nature of the frog skin potential. *Acta physiol. scand.*, **42**, 298–308.

RICK, R., DÖRGE, A. & NAGEL, W. (1975). Influx and efflux of sodium at the outer surface of frog skin. *J. Membrane Biol.*, **22**, 183–196.

SCHULTES, R.E. (1969). Hallucinogens of plant origin. *Science,* **163**, 245–254.

SEPULVEDA, F.V. & ROBINSON, J.W.L. (1974). Harmaline, a potent inhibitory of sodium dependent transport. *Biochim. biophys. Acta,* **373**, 527–531.

USSING, H.H. (1960). The frog skin potential. *J. gen. Physiol.,* **43**, 135–147.

USSING, H.H. & WINDHAGER, E.E. (1964). Nature of shunt path and active sodium transport path through frog skin epithelium. *Acta physiol. scand.,* **61**, 484–504.

ZEISKE, W. & LINDEMANN, B. (1974). Chemical stimulation of Na^+ current through the outer surface of frog skin epithelium. *Biochim. biophys. Acta,* **352**, 323–326.

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