J. exp. Biol. **105**, 83–94 (1983)
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PARDAXIN PRODUCES SODIUM INFLUX IN THE TELEOST GILL-LIKE OPERCULAR EPITHELIA

BY NAFTALI PRIMOR*

Department of Physiology and Biophysics, New York University Medical Center, 550 First Avenue, New York, New York 10016, U.S.A. and *Osborn Laboratories of Marine Sciences, New York Aquarium, New York Zoological Society, Brooklyn, New York 11224, U.S.A.*

(*Received 16 August 1982—Accepted 31 January 1983*)

SUMMARY

1. Transport by the gill-like opercular epithelium of the teleost, *Fundulus heteroclitus*, was affected by pardaxin, a protein that is toxic to fish, isolated from the Red Sea flatfish *Pardachirus marmoratus*.

2. Administration of pardaxin to the mucosal (seawater) side of the isolated short-circuited opercular epithelium, caused a transient stimulation of the active transport of ions (Isc), followed by an inhibition. The Isc stimulation was abolished by ouabain or/and in Na^+-free Ringer but not in Cl^--or HCO_3^--Ringer. When applied to the serosal (blood) side, pardaxin did not affect the Isc.

3. Pardaxin produced a net transient Na^+ current from the mucosal side to the serosal side of 2·2 μequiv $cm^{-2} h^{-1}$. It is concluded that this Na^+ influx caused the Isc stimulation. The influx is suggested to be the mechanism of pardaxin's toxicity in fish.

INTRODUCTION

The Red Sea flatfish *Pardachirus marmoratus* exudes a fluid from more than 200 glands located along its dorsal and anal fins (Clark & Chao, 1973) which is toxic to fish (Clark & George, 1979). The toxic component is a protein named pardaxin (Primor, Parness & Zlotkin, 1978). It is constructed of a monomeric chain, with a helical structure and four disulphide bridges (Primor & Tu, 1980).

Pardaxin probably acts on the gills in both teleosts and elasmobranchs and its toxicity is elevated in fish preadapted to a medium of high salinity. Furthermore, its toxicity decreases significantly when injected into the fish (Primor, Sabnay, Lavie & Zlotkin, 1980*a*; Primor, Zadunaisky, Murdaugh & Forrest, 1980*b*).

The mode of action of pardaxin is examined here by studying its effect upon transport by the opercular epithelium of the killifish *Fundulus heteroclitus*, whose ion-osmoregulatory properties closely resemble those of teleost gills (Degnan, Karnaky & Zadunaisky, 1977).

* Present address: Osborn Laboratories of Marine Sciences, New York Aquarium, New York Zoological Society, Brooklyn, New York 11224 U.S.A.

Key words: Pardaxin, fish toxin, ion-osmoregulation, skin epithelium.

MATERIALS AND METHODS

Pardachirus marmoratus fish (Pisces; Soleidae) were collected from The Red Sea (Eilat, Israel). *P. marmoratus* secretion was extracted by the method described by Clark & Chao, (1973). Pardaxin (PX), the ichthyotoxic component isolated from the secretion was obtained according to Primor *et al.* (1978). The molecular weight was estimated by SDS gel electrophoresis and found to be correspondent to $M_r = 13\,100$ (N. Primor, in preparation).

Killifish, *Fundulus heteroclitus*, of both genders were collected from the estuaries near the Osborn Laboratories of Marine Sciences, New York Aquarium, Coney Island, Brooklyn, New York. They were adapted to artificial sea water (Utility Chemical Co., Paterson, New Jersey) as described by Karnaky, Kinter, Kinter & Stirling (1976). The experiments were performed at room temperature (22–24 °C) for a year.

Tissue preparation

The opercular epithelium was prepared and mounted in a Lucite chamber as previously described (Degnan *et al.* 1977; Degnan & Zadunaisky, 1980*a*). Briefly, the opercular epithelium tissue was placed between two disks of polymerized Sylgard (Dow Corning, Midland, Michigan) with centrally located circular apertures. Two epithelia from the same fish were mounted in matching chambers within 10–20 min of removing the fish from the seawater tank.

Solutions

The Ringer contained (in mM): NaCl, 135·0; KCl, 2·5; $MgCl_2$, 1·0; $CaCl_2$, 1·5; $NaHCO_3$, 16·0; glucose, 5·0 and was gassed with 95 % O_2/5 % CO_2 (pH 7·15). In the ion substitution experiments, equivalent substitution was made of sodium by choline, potassium by sodium, and chloride by methylsulphate (Sigma Chemical Co., St. Louis, Missouri). In HCO_3^--free Ringer experiments the chambers were gassed with air. The sea water used to determine pardaxin's toxicity to *F. heteroclitus* consisted of (in mM): NaCl, 480; KCl, 10; $CaCl_2$, 20; $MgCl_2$, 24; and $MgSO_4$, 28. The lethal dose (LD_{50}) was calculated by the method of Reed & Muench (1938).

Electrical and unidirectional fluxes: measurements and calculations

The procedures for measuring the transepithelial potential difference (p.d.), applying the short-circuit current (Isc), and calculating the transepithelial d.c. resistance (R) or conductance (G_t), have been described previously (Degnan *et al.* 1977). $^{22}Na^+$, $^{36}Cl^-$ and ^{3}H-inulin were obtained from New England Nuclear, Boston, Massachusetts. Concentrated isotope stock solutions were prepared in Ringer and 10–50 μl aliquots, containing 5 μCi of $^{22}Na^+$ or $^{36}Cl^-$ or 20 μCi of ^{3}H-inulin, were added to one side in the matching chambers and allowed to equilibrate for 60 min. Inulin fluxes were measured with Ringer containing 1 mM-inulin (Fisher Scientific, Fair Lawn, N.J.). Samples from the 'cold' side were taken at 10 or 20 min intervals and the activity determined by liquid scintillation. The 'cold' side volume was kept constant by replacing the sample removed for counting with an equal volume of Ringer, or Ringer containing drug.

The serosal to mucosal flux (efflux, J_{sm}) was measured in one preparation while the mucosal to serosal flux (influx, J_{ms}) was measured in the paired preparation. The net Cl^- flux was calculated as the average efflux minus the average influx.

The Na^+ fluxes across the short-circuited opercular epithelium have been shown to be passive and traversing only one rate-limiting barrier (Degnan & Zadunaisky, 1980*a*). This type of behaviour is attributed to movement through the paracellular shunt pathway (Bruus, Kristensen & Larsen, 1976).

The Na^+ permeability is assumed to be independent of the voltage across the membrane. The relationship between the Na^+ unidirectional flux (J_{Na}) and the permeability (P_{Na}) and tissue conductance (G_t) was applied as previously described by Degnan & Zadunaisky (1980*b*) and summarized in Table 3.

RESULTS

The effect of pardaxin on the electrical properties of the short-circuited killifish opercular epithelium

The isolated opercular epithelia of seawater-adapted fish, when bathed on both sides with Ringer, displayed a mean short-circuit current (Isc) of 146·1 $\mu A\,cm^{-2}$, a

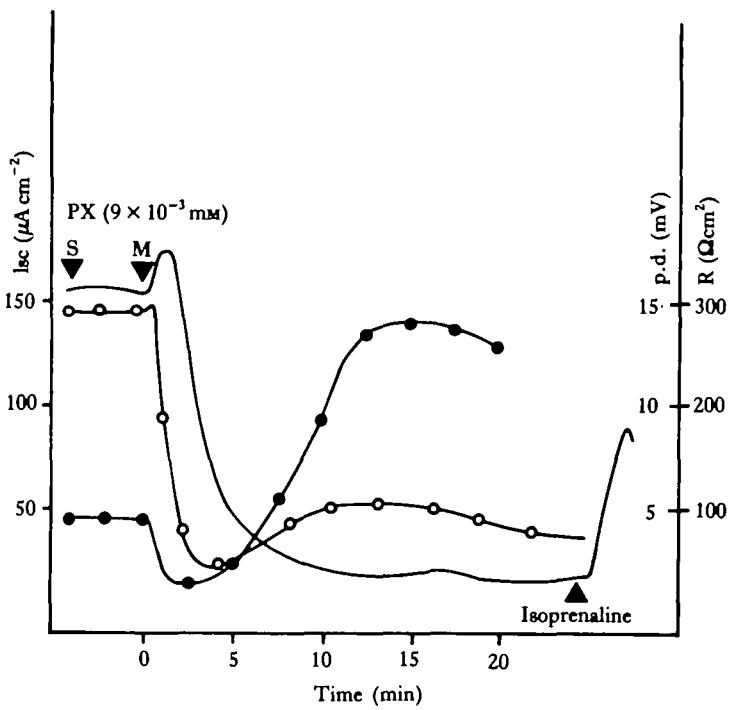

Fig. 1. The effect of pardaxin (PX) administered to the mucosal side (M) and to the serosal side (S) on the electrical properties of the isolated short-circuited opercular epithelium from seawater-adapted *F. heteroclitus* in Cl^- and Na^+-rich Ringer. PX (9×10^{-3} mM) was given on the serosal side. After 5 min it was administered on the mucosal side. Isoprenaline (5×10^{-4} mM) was added to the serosal side. (——), Tracing of continuous short-circuit current (Isc); (○——○), transepithelial potential difference (p.d.); (●——●), calculated resistance (R). Note PX first stimulates and secondarily inhibits the Isc.

Table 1. *The effect of pardaxin on the short-circuit (Isc), transepithelial potential difference (p.d.) and resistance (R) across the isolated opercular epithelia from the seawater-adapted teleost,* Fundulus heteroclitus

Time (min)	Control			Pardaxin 3×10^{-3} mM ($N = 10$)			Pardaxin 6×10^{-3} mM ($N = 20$)			Pardaxin 9×10^{-3} mM ($N = 10$)		
	Isc	p.d.	R	Isc	p.d.	R	Isc	p.d.	R	Isc	p.d.	R
20% change	146·1 ± 18·6	17·5 ± 5·6	119·7	112·1 ± 20·0 −23·2	12·1 ± 3·6 −30·8	107·9 −9·8	22·6 ± 9·6 −84·5	4·9 ± 1·9 −72·0	216·8 +81·1	22·3 ± 4·1 −84·7	4·0 ± 1·1 −77·1	179·3 +49·7
40% change	133·0 ± 20·1	16·1 ± 7·6	121·0	110·6 ± 18·4 −16·8	14·9 ± 5·1 −7·4	134·7 +11·3	18·2 ± 7·8 −86·3	4·2 ± 1·7 −73·9	230·7 +90·6	17·6 ± 5·9 −86·7	3·6 ± 0·9 −77·1	204·5 +69·0
60% change	122·5 ± 17·6	15·2 ± 6·5	124·1	98·1 ± 18·9 −19·9	13·7 ± 4·0 −9·8	139·6 +12·4	16·5 ± 11·5 −86·5	3·5 ± 1·0 −76·9	212·1 +70·9	11·5 ± 3·1 −90·6	3·1 ± 0·7 −79·6	269·5 +117·1
80% change	101·6 ± 21·4	13·6 ± 6·8	133·8	99·6 ± 20·5 −1·9	10·1 ± 3·9 −25·7	101·4 −24·2	14·6 ± 9·7 −85·6	2·9 ± 0·6 −78·6	198·6 +48·4	9·6 ± 3·2 −90·5	2·2 ± 0·5 −83·8	229·1 +71·2

The Isc is given in units of $\mu A\,cm^{-2}$, the p.d. in mV and the resistance in Ωcm^2. Pardaxin was added to the mucosal (seawater) side only. The data are expressed as mean ± S.E.M. and the number of paired experiments are given in parenthesis.

mean transepithelial potential difference (p.d.) of 17·5 mV (serosal side positive), and a mean transepithelial d.c. resistance of 119·7 Ωcm^2 (Table 1).

Pardaxin (PX) at 3×10^{-3} mM ($40\,\mu g\,ml^{-1}$) 6×10^{-3} mM ($80\,\mu g\,ml^{-1}$) and 9×10^{-3} mM ($120\,\mu g\,ml^{-1}$) administered into the mucosal (seawater) side of the opercular epithelium elicited a dual effect on the Isc. At first, the Isc was transiently stimulated, and then, within 20 min inhibited (Fig. 1). At 3×10^{-3} mM the Isc recovered within 80 min, but at higher concentrations the Isc was irreversibly inhibited (Table 1). Following PX action, the epithelium was capable of responding to isoprenaline stimulation (5×10^{-4} mM, on the serosal side). This finding suggests that PX does not destroy the epithelium's response to β-adrenergic activation of Cl^- secretion (Degnan & Zadunaisky, 1979; Mendelson, Cherksey & Degnan, 1981).

To identify the ion(s) involved in the elevation of the Isc, various ions were substituted by others. In Cl^--free Ringer, PX (9×10^{-3} mM) administered to the mucosal side elicited a large transient Isc stimulation. This effect was not observed when PX was added to the serosal side (Fig. 2). In the opercular epithelium, the rate of Cl^- secretion has been shown to be dependent upon the HCO_3^- concentration (Degnan *et al.* 1977). In HCO_3^--free Ringer, PX (9×10^{-3} mM) on the mucosal side produced Isc stimulation similar to that observed in Cl^--free Ringer. PX on the serosal side had no effect. In addition, the presence of 10^{-3} M-acetazolamide (carbonic anhydrase inhibitor) did not depress the PX-induced Isc stimulation in HCO_3^--free Ringer (data not shown). In Na^+-free Ringer, PX (9×10^{-3} mM) on the mucosal side did not stimulate the Isc (data not shown). Removal of K^+ resulted in a decrease of Isc from $180\,\mu A\,cm^{-2}$ to $30\,\mu A\,cm^{-2}$; and addition of PX (9×10^{-3} mM) on the mucosal side then caused an elevation of Isc (Fig. 3).

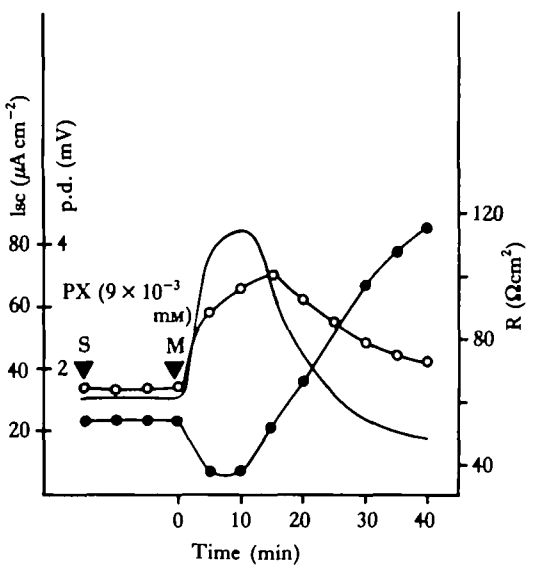

Fig. 2. The effect of pardaxin (PX) administered to the mucosal side (M) and to the serosal side (S) on the electrical properties of the isolated short-circuited opercular epithelium from seawater-adapted *F. heteroclitus* in Cl^--free Ringer. (——), Tracing of continuous short-circuit current (Isc); (○——○), transepithelial potential difference (p.d.); (●——●), calculated resistance (R). Note PX (9×10^{-3} mM) stimulates the Isc only when given on the mucosal side.

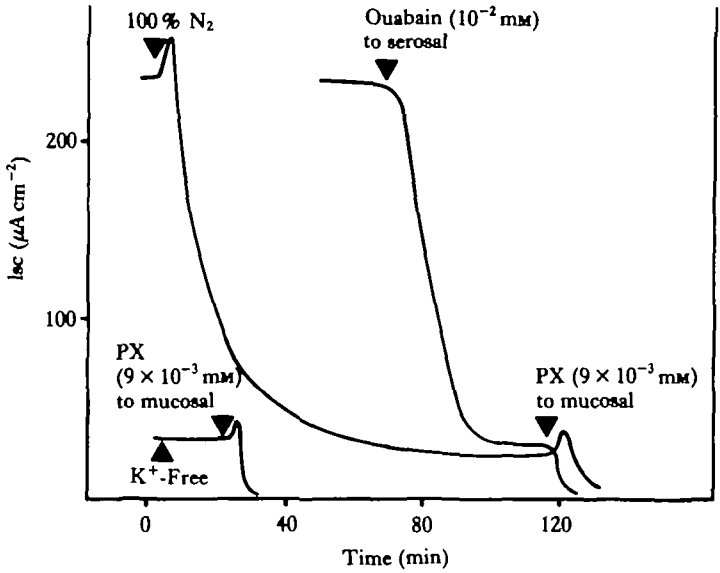

Fig. 3. The effects of ouabain, anoxia and removal of K^+ on pardaxin (PX)-induced short-circuit current (Isc) stimulation in the opercular epithelium from seawater-adapted *F. heteroclitus* in Cl^- and Na^+-rich Ringer. Ouabain (10^{-2} mM) was added to the serosal side. PX (9×10^{-3} mM) was given on the mucosal side. Anoxia was introduced by gassing with 100 % N_2. Note that ouabain prevented the PX-induced Isc stimulation.

Anoxia has been shown to inhibit, by 83 %, the Isc in the operculum (Degnan *et al.* 1977), indicating that the active transport of ions is dependent on metabolic energy. The dual effect of PX (Isc stimulation followed by inhibition) was again observed when the Ringer was gassed with 100 % N_2 instead of 95 % O_2/5 % CO_2.

Pretreatment on the serosal side with ouabain (10^{-2} mM) the classical inhibitor of Na^+, K^+-ATPase, prevented PX (9×10^{-3} mM) on the mucosal side from stimulating the Isc in both the Cl^--Ringer (Fig. 3) and the Cl^--free Ringer (Fig. 4). The most simple interpretation of this experiment is that ouabain abolishes the ion gradient between the cells and the bathing medium and prevents PX from inducing a net sodium influx across the epithelium.

The above results indicate that Na^+ is directly involved in PX-induced active transport stimulation but that Cl^-, HCO_3^- and K^+ are not.

The effect of PX on Cl^-, Na^+ and inulin fluxes across the short-circuited killifish opercular epithelium

In the short-circuited epithelia of seawater-adapted fish, bathed on both sides with Ringer, the mean Cl^- serosal side to mucosal side flux (efflux) was 130·8 μA cm^{-2} and the mean Cl^- mucosal side to serosal side flux (influx) was 28·5 μA cm^{-2}. This resulted in a net Cl^- serosal to mucosal flux of 104·9 μA cm^{-2}. On the other hand, the mean Na^+ efflux was 169·6 μA cm^{-2} and the mean Na^+ influx was 192·9 μA cm^{-2} (Table 2). Despite the relatively large Na^+ fluxes there is no significant net flux (Degnan & Zadunaisky, 1980*a*).

After administration of PX (9×10^{-3} mM) to the mucosal side, the net flux of Cl^- was found to be inhibited by 48·8 % (Table 2). Sodium efflux was inhibited, resulti[illegible]

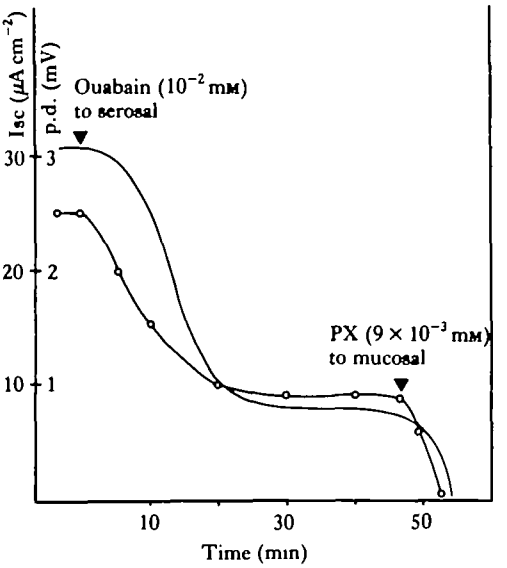

Fig. 4. The effect of ouabain on pardaxin (PX)-induced short-circuit current (Isc) stimulation in Cl^--free Ringer of the isolated opercular epithelium from seawater-adapted *F. heteroclitus*. Following the addition of ouabain (10^{-2} mM) to the serosal side, PX (9×10^{-3} mM) was given to the mucosal side. Note that ouabain prevented the PX-induced Isc stimulation. (——), Tracing of continuous Isc; (O——O), transepithelial potential difference (p.d.).

in a significant ($P = 0{\cdot}005$–$0{\cdot}01$) net sodium influx of $58{\cdot}9\ \mu A\ cm^{-2}$ (Table 2) which is equal to $2{\cdot}2\ \mu equiv\ cm^{-2}\ h^{-1}$ (Table 3).

Sodium permeability (P_{Na}) and conductance (G_{Na}) were calculated from J_{Na} values as obtained for sodium unidirectional fluxes. The tissue conductance (G_t) was mostly due to the contribution of the sodium conductance (Table 3). Therefore the effect of PX on the tissue conductance reflected mostly a change in G_{Na}. The J_{Na}, P_{Na} and G_{Na} for 20 min and 40 min were considerably larger from the mucosal to the serosal side than the opposite direction (Table 3).

Gill permeability was assessed with inulin as has been used for teleosts (Kirschner, 1980*a*; Isaia, 1982). The unidirectional inulin fluxes were found to be 4–5 orders of magnitude lower than the sodium fluxes. Within 20 min after PX application, the inulin fluxes reached a maximum of $2{\cdot}2 \times 10^{-3}\ \mu equiv\ cm^{-2}\ h^{-1}$ which is 10–13 times higher than the control ($0{\cdot}16 \times 10^{-3}\ \mu equiv\ cm^{-2}\ h^{-1}$) and then decayed. They were still 3·3 times higher than the control after 50 min (Table 4).

Toxicity of PX to the teleost F. heteroclitus *adapted to sea water and Ringer medium*

For a period of 3 weeks, *F. heteroclitus* fish were adapted to artificial sea water and to the fish Ringer (Materials and Methods). The lethal dose (LD_{50}) of PX using 40 *F. heteroclitus* (weighing 0·2–0·3 g) adapted to sea water was found to be $30{\cdot}0\ \mu g\ ml^{-1}\ g\ body\ weight^{-1}$, as determined after 20 min. The LD_{50} in fish adapted to the fish Ringer was much higher at $500\ \mu g\ ml^{-1}\ g^{-1}$. Thus the PX toxicity in *F. heteroclitus* is dependent upon the medium concentration as previously noted for *Aphanius dispar* (Primor *et al.* 1980*a*) and the effects elicited by PX in the operculum preparation in Ringer solution were produced by a sub-lethal dose.

Table 2. *The effect of pardaxin (PX) on unidirectional $^{36}Cl^-$ and $^{22}Na^+$ fluxes and the short-circuit current (Isc) across the isolated opercular epithelia from the seawater-adapted teleost,* Fundulus heteroclitus

	$^{36}Cl^-$ ($N = 10$)			($N = 20$)	$^{22}Na^+$ ($N = 10$)	
	Efflux	Influx	Net flux	Isc	Efflux	Influx
				$\mu A\ cm^{-2}$		
Mean control	130·8 ± 15·6	28·5 ± 1·0	104·9 ± 16·0	126·5 ± 16·8	169·6 ± 12·7	192·9 ± 12·8
20 min after PX	80·1 ± 20·5	26·4 ± 1·7	53·7 ± 21·7	22·6 ± 9·6	147·1 ± 11·4	206·0 ± 24·2
% change (compared to control)	−38·8	−7·3	−48·8	−84·5	−13·2	+6·8
P	0·01–0·02	0·40–0·50	0·25–0·05	0·001	0·05–0·10	0·40–0·50
% change, influx to efflux						40·0
P						0·005–0·01

The fluxes are given in units of current (I_{Cl}, I_{Na}) for comparison with the Isc. PX was added to the mucosal side at a concentration of $9·1 \times 10^{-3}$ mM. The data are expressed as mean ± S.E.M. and the number of paired experiments are given in parenthesis.

Table 3. *The effect of pardaxin (PX) on Na^+ fluxes (J_{Na}), permeabilities (P_{Na}) and tissue conductances (G_t) across short-circuited opercular epithelia from the seawater-adapted teleost,* Fundulus heteroclitus

	J_{Na}		P_{Na}		G_{Na}		G_t	G_{Na}/G_t
	Efflux	Influx	Efflux	Influx	Efflux	Influx		
	(μequiv $cm^{-2} h^{-1}$)		(10^{-6} cm s^{-1})		(mmho cm^{-2})			
Control	6·32	7·19	15·35	17·45	6·64	7·56	7·22	0·91
20 min after PX	5·48	7·68*	13·30	18·68	5·68	8·07	5·02	1·17
40 min after PX	3·57	5·38	8·71	13·07	3·75	5·65	3·03	1·23
60 min after PX	3·14	3·60	7·66	8·77	3·30	3·78	1·83	1·80
80 min after PX	2·44	2·99	6·04	7·29	2·53	3·14	1·46	1·73

The results are means calculated from unidirectional $^{22}Na^+$ fluxes.
* The difference between J_{Na} influx and J_{Na} efflux is a net flux of 2·2 μequiv $cm^{-2} h^{-1}$ ($P \simeq 0{\cdot}005$–$0{\cdot}01$).

Table 4. *The effect of pardaxin on ^3H-inulin fluxes across short-circuited isolated opercular epithelia from the seawater-adapted teleost,* Fundulus heteroclitus

	Inulin	
	Efflux	Influx
	(μequiv $cm^{-2} h^{-1}$) $\times 10^{-3}$	
Mean control	0·165 ± 0·03	0·164 ± 0·04
10 min	1·368 ± 0·53	0·861 ± 0·42
% Change	741·3	425·1
P	0·05–0·10	0·01–0·02
20 min	2·36 ± 0·86	1·90 ± 0·69
% Change	1315·9	1061·2
P	0·05–0·10	0·05–0·10
30 min	1·64 ± 0·52	1·55 ± 0·41
% Change	895·2	1001·4
P	0·025–0·05	0·05–0·10
50 min	0·55 ± 0·26	0·54 ± 0·15
% Change	234·5	230·5
P	0·10–0·20	0·025–0·05
60 min	0·60 ± 0·23	0·52 ± 0·17
% Change	265·8	218·2
P	0·05–0·10	0·05–0·10

The data are expressed as μequiv of inulin and are mean ± S.E.M. of five paired experiments. Pardaxin was added to the mucosal side only at a concentration of 9×10^{-3} mM.

DISCUSSION

Pardaxin applied to the mucosal side of the opercular epithelium caused a transient stimulation of active ion transport and then an inhibition, as indicated by the Isc (Fig. 1). The mechanism of the stimulation was found to be sodium-dependent. Examination of the $^{22}Na^+$ fluxes (Table 2) shows that PX elicited a significant ($P = 0{\cdot}01$–$0{\cdot}005$) net Na^+ influx of about 60 μA cm^{-2}. A similar increase in current was produced by pardaxin in the absence of chloride (Fig. 2). Examination of Na^+ (Table 3), inulin (Table 4), and Isc stimulation (Fig. 2) reveals a transient action in spite of the continuous presence of PX. The finding that ouabain (10^{-2} mM added to the serosal side) abolished the PX-induced Isc stimulation (Figs 3, 4) supports the view that the

net movement of Na^+ across the epithelium increased the Isc. Degnan & Zadunaisky (1980*a*) have shown that in opercular epithelium Na^+ movements are passive (diffusional) and paracellular while PX stimulation is active and ouabain-sensitive. Further, the stimulation of Isc strongly suggests that the induced Na^+ influx is cellular rather than paracellular. Therefore, this result may indicate that PX acts on or produces an active cellular Na^+ pathway.

In the opercular epithelium the Isc is chiefly produced by net Cl^- secretion (Degnan *et al.* 1977). PX inhibition of the Isc following its initial stimulation, may thus have resulted from inhibition of Cl^- secretion as indicated by inhibition of net flux (Table 2).

Inulin is believed to cross the teleost gill by a paracellular route among the chloride cells (Kirschner, 1980*a*; Isaia, 1982). The increase in inulin permeability induced in the killifish opercular epithelium by PX (Table 4) may thus indicate an increased paracellular leak. However, PX might also damage the membranes of the epithelial cells, since it increases permeability of red cells (Primor & Lazarovici, 1981), the viral envelope (Pal, Bernholz & Wagner, 1981*a,b*), and lipid bilayers (Moran, Primor & Zlotkin, 1977; Korchak, 1979) and vesicles (Zlotkin & Bernholz, 1983). An analysis of $^{22}Na^+$ and $^{36}Cl^-$ fluxes (Table 2) shows, however, that during the first 20 min there is no increase in the unidirectional permeability. From 40 min to 80 min after adding PX, the Na^+ conductance (as calculated from the unidirectional fluxes) dropped gradually (Table 3). This finding is correlated with an increase in transepithelial resistance (Fig. 1). While PX administered to the serosal side did not elicit Isc stimulation, it did not inhibit Isc stimulation when PX was later added to the mucosal side (Figs 1, 2).

The results indicate that PX did not cause a substantial leak to Na^+ or to Cl^- but a transient Na^+ influx (Table 3). The increase in inulin permeability (Table 4) is about three orders of magnitude lower than the value of the net Na^+ influx created by PX (Table 4). Therefore an increase in paracellular or cellular leak through necrotic cells is only a small fraction of its activity. The most marked effect is the production of a net Na^+ influx.

The mode of action of PX in molecular terms remains unknown. However, it should be noted that it has a surfactant effect (Primor, Zadunaisky & Tu, 1983; Zlotkin & Bernholz, 1983).

The Isc was stimulated by PX only when administration was made on the mucosal side. Similarly, during PX toxicity tests in dogfish, an inhibition of the spiracular rate and an extensive struggle, as well as the production of a transient leak to urea and sodium, were elicited when PX was administered to the medium and were not observed when it was injected into the dorsal artery (Primor *et al.* 1980*a,b*).

In teleosts the serosal side of the branchial epithelium is known to be directly involved in active transport of ions (Pic, 1978; Kirschner, 1980*b*; Karnaky *et al.* 1976). However, ion and acid-base modulation are thought to be located on the mucosal side of the branchial chloride cells (Girard & Payan, 1980; Perry, Haswell, Randall & Farrell, 1981). In addition, the apical membrane is involved in active Na^+ secretion (Bradley, 1981). It is suggested that PX interrupts the process of Na^+ extrusion causing its inhibition which finally leads to a net increase in Na^+ influx.

In a high salinity medium, the Na^+ influx can be expected to be larger than in Ringer solution. PX-enhanced toxicity in a high salinity medium further supports the notion that a net Na^+ influx is the mechanism of PX toxicity in fish.

This author is grateful to Dr Kevin Degnan for introducing me to the operculum preparation techniques and valuable discussions. Appreciation is expressed to Dr Jose A. Zadunaisky for his help in performing part of this work in his laboratory. I would like to thank Dr George D. Ruggieri for providing some of the fish for these studies and to Esta Van Tuyl for excellent typing of the manuscript. Financial support from: The Department of Defense, Office of Naval Research, Research Grants No. N00014-81-K-0667 and N00014-82-C-0435 is acknowledged.

REFERENCES

BRADLEY, T. J. (1981). Improved visualization of apical vesicles in chloride cells of fish gills using an osmium quick-fix technique. *J. exp. Zool.* **217**, 185–198.

BRUUS, K., KRISTENSEN, P. & LARSEN, E. H. (1976). Pathway for chloride and sodium transport across toad skin. *Acta physiol. scand.* **97**, 31–47.

CLARK, E. (1974). The Red Sea's shark proof fish. *Natl Geogr.* **146**, 718–728.

CLARK, E. & CHAO, S. (1973). A toxic secretion from the Red Sea flatfish *Pardachirus marmoratus* (Lacepede). *Sea Fish Res. Haifa Bull.* **60**, 53–56.

CLARK, E. & GEORGE, A. (1979). Toxic soles, *Pardachirus marmoratus* from Red Sea and *P. pavoninus* from Japan, with notes on other species. *Env. Biol. Fish.* **4**, 103–123.

DEGNAN, K. J., KARNAKY, J. J. & ZADUNAISKY, J. A. (1977). Active chloride transport in the *in vitro* opercular skin of a teleost (*Fundulus heteroclitus*), a gill-like epithelium rich in chloride cells. *J. Physiol., Lond.* **271**, 155–191.

DEGNAN, K. J. & ZADUNAISKY, J. A. (1979). Open-circuit sodium and chloride fluxes across isolated opercular epithelia from the teleost *Fundulus heteroclitus*. *J. Physiol., Lond.* **294**, 483–495.

DEGNAN, K. J. & ZADUNAISKY, J. A. (1980*a*). Passive sodium movements across the opercular epithelium: the paracellular shunt pathway and ionic conductance. *J. Membrane Biol.* **55**, 175–185.

DEGNAN, K. J. & ZADUNAISKY, J. A. (1980*b*). Ionic contributions to the potential and current across the opercular epithelium. *Am. J. Physiol.* **328**, 231–239.

GIRARD, J. P. & PAYAN, P. (1980). Ion exchanges through respiratory and chloride cells in freshwater and seawater-adapted teleoteans. *Am. J. Physiol.* **238**, 260–268.

ISAIA, J. (1982). Effects of environmental salinity on branchial permeability of rainbow trout, *Salmo gairdneri*. *J. Physiol., Lond.* **326**, 297–307.

KARNAKY, K. J., KINTER, L. B., KINTER, W. B. & STIRLING, C. E. (1976). Teleost chloride cell. II. Autoradiographic localization of gill Na,K-ATPase in killifish *Fundulus heteroclitus* adapted to low and high salinity environments. *J. Cell Biol.* **70**, 157–177.

KIRSCHNER, L. B. (1980*a*). Uses and limitations of inulin and mannitol for monitoring gill permeability changes in the rainbow trout. *J. exp. Biol.* **85**, 202–211.

KIRSCHNER, L. B. (1980*b*). Comparison of vertebrate salt-excreting organs. *Am. J. Physiol.* **238**, 219–223.

KORCHAK, Z. (1979). The electrochemical characteristics of lipid bilayer modified with *Pardachirus* toxin. *Technion-Israel Institute of Technology*, MSc thesis. Haifa, Israel.

MENDELSON, S. A., CHERKSEY, B. D. & DEGNAN, K. J. (1981). Adrenergic regulation of chloride secretion across the opercular epithelium: the role of cyclic AMP. *J. comp. Physiol.* **145B**, 29–35.

MORAN, A., PRIMOR, N. & ZLOTKIN, E. (1977). The effect of *Pardachirus* venom on the conductance of lipid bilayer. *Annual Meeting of the Israeli Pharmacological-Physiological Society*. Nes Ziona, Israel, Abstract.

PAL, R., BERNHOLZ, Y. & WAGNER, R. R. (1981*a*). Pardaxin, a hydrophobic toxin of the Red Sea flatfish, disassembles the intact membrane of vesicular stomatitis virus. *J. biol. Chem.* **256**, 10209–10212.

PAL, R., BERNHOLZ, Y. & WAGNER, R. R. (1981*b*). Transcription of vesicular stomatitis-virus activated by pardaxin, a fish toxin that permeabilized the virion membrane. *J. Virol.* **39**, 641–645.

PERRY, S. J., HASWELL, M. S., RANDALL, D. J. & FARRELL, A. P. (1981). Branchial ionic uptake and acid-base regulation in the rainbow trout, *Salmo gairdneri*. *J. exp. Biol.* **92**, 289–303.

PIC, P. (1978). A comparative study of the mechanism of Na^+ and Cl^- excretion by the gill of *Mugil capito* and *Fundulus heteroclitus*: effects of stress. *J. comp. Physiol.* **123B**, 155–162.

PRIMOR, N. & LAZAROVICI, P. (1981). *Pardachirus marmoratus* (Red Sea flatfish) secretion and its isolated toxic fraction pardaxin: the relationship between hemolysis and ATPase inhibition. *Toxicon* **19**, 573–578.

PRIMOR, N., PARNESS, J. & ZLOTKIN, E. (1978). Pardaxin: The toxic factor from the skin secretion of the flatfish *Pardachirus marmoratus* (Soleidae). In *Toxins: Animal, Plant and Microbial*, (ed. P. Rosenberg), pp. 539–547. New York: Pergamon Press.

PRIMOR, N., SABNAY, I., LAVIE, V. & ZLOTKIN, E. (1980*a*). Toxicity to fish, effect on gill ATPase and gill ultrastructural changes induced by *Pardachirus* secretion and its derived toxin pardaxin. *J. exp. Zool.* **211**, 33–43.

PRIMOR, N. & TU, A. T. (1980). Conformation of pardaxin, the toxin of the flatfish *Pardachirus marmoratus*. *Biochem. biophys. Acta* **626**, 299–306.

PRIMOR, N., ZADUNAISKY, J. A., MURDAUGH, V. H. & FORREST, J. N. (1980*b*). A study of pardaxin toxicity in the dogfish. *Mt. Desert Island Biol. Lab.* **20**, 120–122.

PRIMOR, N., ZADUNAISKY, J. A. & TU, A. T. (1983). Pardaxin, its action, toxicity and structure. In *Sharks Repellents from the Sea: New Perspectives*, (ed. B. J. Zahuranec), pp. 173–184. Washington D.C.: American Association for the Advancement of Science.

REED, L. Y. & MUENCH, H. (1938). A simple method for estimating fifty percent end points. *Am. J. Hyg.* **27**, 493–497.

ZLOTKIN, E. & BERNHOLZ, Y. (1983). Studies on the mode of action of the ichthyotoxin pardaxin. In *Sharks Repellents from the Sea: New Perspectives*, (ed. B. J. Zahuranec), pp. 157–171. Washington D.C.: American Association for the Advancement of Science.