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EFFECTS OF ORTHOVANADATE ON SALT AND WATER EFFLUXES FROM THE GILLS OF SEAWATER EELS, *ANGUILLA ANGUILLA*

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Orthovanadate ($5 \cdot 10^{-7}$ M) perfused through isolated gills at a constant rate increased the perfusion pressure by 40% but inhibited the effluxes of Na^+ and Cl^- by 40%. Water efflux was unaltered. Ouabain (10^{-4} M) and rotenone (10^{-4} M) influenced salt and water effluxes in the same way but did not alter perfusion pressures. Orthovanadate (10^{-5} M) perfused at constant rate increased the pressure nearly 2.5-fold; under these conditions effluxes of Na^+, Cl^- and H_2O were all increased approximately 2.5-fold.

Marine salt secreting epithelia including the gills of teleost fishes and the rectal glands of elasmobranchs depend critically on ($Na^+ + K^+$)-dependent ATPase for their correct functioning [1]. Adenyl cyclase also appears to be involved in salt secretion by these epithelia since secretion is greatly enhanced and sustained by administration of either dibutyryl cyclic AMP or theophylline [2,3]. In recent years orthovanadate has been established as a powerful inhibitor of ($Na^+ + K^+$)-depedent ATPase [4–6] and as a stimulator of adenyl cyclase [7,8]. In principle, therefore, vanadate could either inhibit or stimulate salt secretion by marine teleost gills. The exact prediction of the effects of vanadate on gills is also complicated by the powerful vasoconstricting effects of the anion on gills [9,10].

Isolated, perfused heads were prepared from sea water-adapted eels, *Anguilla anguilla*, by methods detailed previously [9–11]. Briefly, aerated sea water was passed across the outer surfaces of the gills at a rate of 1 l/min while a bicarbonate Ringer was perfused internally at a rate of 1 ml/min. The Ringer contained 144 mM NaCl, 2 mM K_2SO_4, 26 mM $NaHCO_3$, 2 mM Na_2HPO_4, 0.4 mM KH_2PO_4, 0.63 mM $CaCl_2$, 2 mM $MgSO_4$, 1.0 g/l of glucose, 20 g/l of polyvinylpyrrolidone (PVP-40), 10^{-6} M adrenaline and 5 000 units/l of heparin. The Ringer was gassed continuously with air/CO_2 (98 : 2, v/v) giving a pH of 7.6 and the entire system maintained at 15°C. All solutions were passed through a 0.22 μM Millipore filter before admission to the gills and changes of perfusing solution were made via a three-way tap so as to avoid interrupting perfusion. Perfusion pressure through the gills was continuously monitored using a pressure transducer (Searle Instrument, Harlow, U.K.) in contact with the perfusion fluid immediately before it entered the gills.

To measure salt and water effluxes, the internal Ringer contained $^{24}Na^+$ (10 μCi/ml), $^{36}Cl^-$ (5 μCi/ml) and ^3H_2O (1 μCi/ml). Thirty min after starting perfusion, the external sea water was sampled every 2 min over a 60-min period. Samples were radioassayed for ^{24}Na by conventional gamma spectrometry. ^{24}Na activity was allowed to decay for one week and the samples radioassayed for ^{36}Cl and tritium by conventional liquid scintillation spectrometry. Count rates in the external sea water were converted to μequivalents of Na^+ and Cl^- and mmol

of H_2O secreted by reference to measured specific radioactivities in the internal Ringer. Plots of effluxes in μequivalents or mmoles against time were constructed and regression lines fitted by the method of least mean squares. Where inhibitors were tested, regression lines were constructed before and after addition, and the degree of inhibition measured by comparing the respective slopes using an unpaired *t*-test.

Effluxes of ^{24}Na, $^{36}Cl^-$ and ^3H_2O across the gills of sea water-adapted eels in the present work were linear over the 60-min test period. In a series of experiments, the rates recorded (means ± S.D.) were 138.4 ± 69.6 ($n = 6$) and 145.8 ± 85.3 ($n = 6$) μequiv./h/100 g fish for Na^+ and Cl^-, respectively, and 175.8 ± 67.9 ($n = 6$) mmol/h/100 g fish for H_2O. That is, the effluxes of Na^+ and Cl^- are the same and, on a molar basis, the water efflux is some 500 times the salt ($Na^+ + Cl^-$) efflux.

Table I shows that ouabain (10^{-4} M) inhibits the effluxes of both Na^+ and Cl^- by approx. 40% without changing either the water efflux or the perfusion pressure. A similar result is obtained by perfusing rotenone (10^{-4} M). Thus, about one half of the salt outflux from perfused sea water gills is dependent on the operation of ($Na^+ + K^+$)-dependent ATPase (inhibited by ouabain) and the provision of ATP generated by terminal respiration (inhibited by rotenone).

Since orthovanadate ($5 \cdot 10^{-7}$ M) also inhibits both Na^+ and Cl^- effluxes by about 50% without inhibiting water efflux (Table I), it is reasonable to conclude that the anion is inhibiting ($Na^+ + K^+$)-dependent ATPase. The ($Na^+ + K^+$)-dependent ATPase in isolated perfused gills is thus much more sensitive to orthovanadate than to ouabain a finding already noted for the enzyme partially purified from eel gills [12]. While no evidence is available as yet concerning the activity of adenyl cyclase in marine salt secreting epithelia or its sensitivity to vanadate, the adenyl cyclases of mammalian heart and adipose tissue are significantly less sensitive to vanadate than are the ($Na^+ + K^+$)-dependent ATPases of mammalian kidney or marine teleost gills [7,8,5,12]. It appears certain that orthovanadate perfused through gills at $5 \cdot 10^{-7}$ M affects the ($Na^+ + K^+$)-dependent ATPase much more than the adenyl cyclase so that salt efflux is inhibited.

When higher concentrations of orthovanadate (10^{-5} M) are perfused through the isolated gills at a constant rate, the rise in perfusion pressure seen at $5 \cdot 10^{-7}$ M vanadate now becomes more intense (Table I). The final perfusion pressure is nearly 2.5-times the starting value and probably exceeds physiological limits. Obviously the ($Na^+ + K^+$)-dependent ATPase in the gills is inhibited under these conditions. Nevertheless the effluxes of Na^+, Cl^- and H_2O are all increased by approx. 2.5-fold. The gills do not appear to be ruptured by the high perfusion pressures induced by 10^{-5} M vanadate since obvious effluxes of dyes such as Evans Blue are not seen under these conditions. A more likely explanation is that the observed effluxes of Na^+, Cl^- and H_2O under high perfusion pressures reflect

TABLE I

EFFECTS OF INHIBITORS ON THE EFFLUXES OF SODIUM AND CHLORIDE IONS AND WATER FROM THE ISOLATED PERFUSED GILLS OF EELS ADAPTED TO SEA WATER

Data are expressed as mean percentages ±S.D. ($n = 6$). Absolute flux values for controls (100% values) are quoted in the text.

Inhibitor	Fluxes (%)			Perfusion pressure (mm Hg)
	Na^+	Cl^-	Water	
None (control)	100	100	100	30.3 ± 1.7
Ouabain, 10^{-4} M	60.2 ± 15.2 *	70.2 ± 5.4 *	109.2 ± 8.1	30.3 ± 1.7
Rotenone, 10^{-4} M	52.2 ± 19.6 *	47.9 ± 5.4 *	93.4 ± 12.0	30.3 ± 1.7
Orthovanadate, $5 \cdot 10^{-7}$ M	57.5 ± 10.8 *	50.0 ± 10.8 *	82.0 ± 20.1	42.0 ± 4.4 *
Orthovanadate, 10^{-5} M	253 ± 32 *	251 ± 56 *	213 ± 42 *	70.6 ± 9.8 *

* Significantly different from controls, $P < 0.01$.

pressure-driven movements of salt and water outwards across the epithelium via the inter- or paracellular route between adjacent chloride cells. These cells are known to be linked together by single-stranded junctions that probably account for the high salt permeability of sea water gills and are probably the route through which salt is secreted even under conditions of normal perfusion pressure [13–15,1]. Similar increases in salt and water effluxes across gills have been noted in isolated, perfused gills of sea water-adapted trout vasoconstricted by ouabain (10^{-5} M) [16]. That isolated perfused eel gills are not vasoconstricted in the present work by even higher concentrations of ouabain (10^{-4} M) may reflect different sensitivities of the two species to ouabain and, even more so, known differences in their gill haemodynamics [17,18]. Biochemical events underlying vasoconstriction of teleost gills by either ouabain or orthovanadate remain to be studied.

We note finally that the inhibition of salt secretion by gills brought about by low concentrations of orthovanadate here is fundamentally the same as the marked natriuresis induced by vanadate perfused through rats [19]. While vanadate undoubtedly influences cardiovascular processes [8,20,9,10], it appears that osmoregulatory processes are more sensitive to the anion. Should vanadate be involved in the physiological regulation of ($Na^+ + K^+$)-dependent ATPase it is probable that this regulation will be most highly developed in the osmoregulating epithelia including mammalian kidney and the marine salt secreting epithelia.

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