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# VANADATE-INDUCED INHIBITION OF SODIUM TRANSPORT AND OF SODIUM-INDEPENDENT ANION TRANSPORT IN TURTLE BLADDER

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## Summary

Vanadate in the serosal bathing fluid of turtle bladders inhibits the $Na^+$ moiety of the short-circuiting current ($I_{sc}$), the anion ($Cl^-$, $HCO_3^-$) moiety of $I_{sc}$, and net $Cl^-$ flux. Since the anion transport is $Na^+$-independent and ouabain-insensitive, its inhibition by vanadate is uniquely different from the well known vanadate-induced inhibition of ($Na^+$+$K^+$)-ATPase and $Na^+$ transport-dependent anion movement of some other epithelia. Vanadate also generates damped oscillations in the bladders' electrical parameters, an unusual effect by an ion in epithelial systems.

Vanadate (V in the +5 oxidation state) possesses a striking natriuretic and diuretic action [1], presumably related to its inhibition of both ($Na^+$+$K^+$)-ATPase [2, 3] and vasopressin-sensitive, cyclic AMP-dependent $Na^+$ and water transport [4]. The recent report by Cantley et al. [5] that vanadate enters the red cell via a disulfonic stilbene-sensitive pathway suggested to me that this oxyanion might interfere with anion transport in the turtle bladder, a well known model epithelium that possesses disulfonic stilbene-inhibitable anion-selective pathways [6—8]. The present report describes some properties of the vanadate-induced inhibition of anion and $Na^+$ transport across bladders of *Pseudemys scripta* turtles.

Methods for evaluating transepithelial potential (*PD*), short-circuiting current ($I_{sc}$), d.c. resistance ($R$), and $^{36}Cl^-$ fluxes have been described [7, 9]. For studies of $Na^+$ transport, paired hemi-bladders were bathed on both surfaces by identical $Na^+$ Ringer solutions; for studies on anion transport, 0.2 mM ouabain was present in the serosal fluid. Sodium metavanadate (Fisher-

Scientific) was used at concentrations of 0.1 and 1 mM. Under the experimental conditions used, the predominant anionic species of vanadate presumably was $H_2VO_4^-$ [10].

Table I shows the effect of vanadate on 13 bladders bathed by $Na^+$ media without exogenous $HCO_3^-$. $I_{sc}$, which under these bathing conditions approximates net reabsorption of $Na^+$ [11—13], was decreased by 65.8% in 2 h. Not shown is that the onset of inhibition consistently occurred within 2—5 min after vanadate addition, but that the rate of decline in the measured parameters varied widely (half-time of 84 ± 13 min). No inhibition of $I_{sc}$ was observed at 0.1 mM vanadate. In five control bladders the time-dependent decay in $I_{sc}$ was 4.6 ± 1.4% per h.

TABLE I

EFFECT OF VANADAT ON *PD*, $I_{sc}$ AND *R* OF BLADDERS IN $HCO_3^-$-FREE $Na^+$ BATHING MEDIUM

Mean values ± S.E. ($n$ = 13) of *PD*, $I_{sc}$, and *R* and of the percentage changes in these parameters 2 h after serosal addition of 1 mM vanadate. Positive values of *PD* and $I_{sc}$ indicate that serosa is electropositive to mucosa. Area of exposed tissue, 1.5 $cm^2$. Composition of bathing fluid (mM): NaCl, 83.5; $Na_2SO_4$, 10.0; KCl, 4; $MgSO_4$, 0.8; $CaSO_4$, 2.0; $K_2HPO_4$, 0.65; $KH_2PO_4$, 0.10; glucose, 11; osmolality was adjusted to 220 mosM/kg with sucrose; final pH, 7.6 ± 0.1; aspirated with $H_2O$-saturated 100% $O_2$.

| Condition | *PD* (mV) | $I_{sc}$ ($\mu$A) | *R* (k$\Omega$) |
|---|---|---|---|
| Before vanadate | 67.5 ± 4.1 | 108.0 ± 8.1 | 0.56 ± 0.04 |
| After vanadate | 21.6 ± 3.3 | 40.2 ± 6.2 | 0.58 ± 0.06 |
| $\Delta$(%)* | —69.6 ± 4.3 | —65.8 ± 4.5 | —1.4 ± 9.1 |
| $P(\Delta=0)$ | $P < 0.001$ | $P < 0.001$ | $P > 0.8$ |

*Note that values of $\Delta$(%) are means ± S.E. of $n$ individual percentage changes in the designated electrical parameters after addition of vanadate. Statistical significance of $\Delta$(%) was calculated by the Student's $t$-test.

Table II shows the effect of vanadate on *PD*, $I_{sc}$, and *R* of 14 bladders (seven pairs of mated hemi-bladders) bathed by $Na^+(Cl^- + HCO_3^-)$ media plus serosal ouabain. $I_{sc}$, which under these bathing conditions is reversed in direction and approximates the algebraic sum of the conductive flows of $Cl^-$ and $HCO_3^-$ [12, 14, 15], was decreased by 72.2% in 2 h. Other results common to both $Na^+$-dependent (Table I) and ouabain-insensitive moieties (Table II) of *PD* and $I_{sc}$ were the following: (i) washing of the serosal surface or exposure to 2 mM norepinephrine [2] failed to restore these parameters to control values; (ii) 1 mM vanadate in the mucosal fluid for at least 1 h failed to decrease *PD* and $I_{sc}$.

Because of the possibility of a non-conductive $Cl^-$-$HCO_3^-$ exchange component of $Cl^-$ transport [16, 17] which would not contribute to $I_{sc}$, $^{36}Cl^-$ fluxes were measured in five of the mated pairs of hemi-bladders of Table II. The measured and calculated $Cl^-$ flux parameters are shown in Table III. The major effect was a 53.7% decrease in the M-to-S flux (M, mucosa; S, serosa). Since the S-to-M fluxes were low relative to the M-to-S fluxes, the changes in the S-to-M fluxes contributed comparatively little to the changes in the net M-to-S $Cl^-$ flux. The effects of time-dependent

TABLE II

EFFECT OF VANADATE ON *PD*, $I_{sc}$, AND *R* OF OUABAIN-TREATED BLADDERS IN $Na^+$ ($Cl^-$ + $HCO_3^-$) BATHING MEDIUM

Mean values ± S.E. ($n$ = 14, seven mated pairs of hemi-bladders) of *PD*, $I_{sc}$, and *R* and of percentage changes in these parameters 2 h after serosal addition of 1 mM vanadate. Negative values of *PD* and $I_{sc}$ indicate that serosa is electronegative to mucosa. Area of exposed tissue, 1.5 $cm^2$. Composition of bathing fluid (mM): NaCl, 21; $NaHCO_3$, 20; $Na_2SO_4$, 30; KCl, 4; $MgSO_4$, 0.8; $CaSO_4$, 2.0; $K_2HPO_4$, 0.65; $KH_2PO_4$, 0.1; glucose, 11; osmolality was adjusted to 220 mosM/kg with sucrose; final pH 7.6 ± 0.1; aspirated with $H_2O$-saturated 98% $O_2$/2% $CO_2$; 0.2 mM ouabain in serosal fluid. Statistical definitions given in Table I.

| Condition | *PD* (mV) | $I_{sc}$ ($\mu$A) | *R* (K$\Omega$) |
|---|---|---|---|
| Before vanadate | −38.3 ± 6.2 | −31.2 ± 5.4 | 1.2 ± 0.1 |
| After vanadate | −8.2 ± 2.3 | −9.0 ± 3.0 | 0.9 ± 0.1 |
| Δ(%) | −75.1 ± 5.3 | −72.2 ± 4.9 | −21.5 ± 4.6 |
| *P* (Δ=0) | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |

TABLE III

EFFECT OF VANADATE ON $Cl^-$ FLUXES ACROSS OUABAIN-TREATED BLADDERS IN $Na^+$ ($Cl^-$ + $HCO_3^-$) BATHING MEDIUM

Mean values ± S.E. ($n$ = 5) of the measured $Cl^-$ fluxes and calculated net fluxes and flux ratios of five pairs of mated hemi-bladders in Table II. Experimental conditions given in Table II. Statistical definitions given in Table I. The value of $Cl^-$ flux before vanadate is the steady-state level during the two 30 min sampling periods immediately before addition of vanadate; the value of $Cl^-$ flux after vanadate is that obtained during the two sampling periods 90—150 min after the addition of vanadate (Ref. 7). Net $Cl^-$ fluxes and flux ratios were calculated from the individual flux differences and flux ratios of the five pairs of mated hemi-bladders. Flux values are for 1.5 $cm^2$ of tissue area.

| Condition | M-S $Cl^-$ flux ($\mu$A) | S-M $Cl^-$ flux ($\mu$A) | Net M-S $Cl^-$ flux ($\mu$A) | Flux ratio (M-S/S-M) |
|---|---|---|---|---|
| Before vanadate | 27.8 ± 4.3 | 2.6 ± 0.4 | 25.1 ± 5.8 | 10.1 ± 2.0 |
| After vanadate | 13.0 ± 2.8 | 4.6 ± 0.5 | 8.0 ± 3.6 | 2.5 ± 0.7 |
| Δ(%) | −53.7 ± 4.8 | +78.2 ± 15.9 | −70.8 ± 6.8 | −73.8 ± 5.0 |
| *P*(Δ=0) | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |

changes in $Cl^-$ fluxes were checked in four pairs of mated hemi-bladders. While the M-to-S fluxes in the vanadate-treated hemi-bladders decreased by 49.6 and 59.7%, those of the untreated hemi-bladders decreased by 2.5 and 7.4%, respectively. The increases in the S-to-M fluxes of treated bladders (78.5 and 39.1%) were similar to those of untreated bladders (33.7 and 80.7%, respectively).

Since net $Cl^-$ reabsorption (Table III) could account for 80% of $I_{sc}$ (Table II), the effect of vanadate on $HCO_3^-$ reabsorption, defined as $I_{sc}$ minus the net $Cl^-$ flux [12, 14, 15], could not be accurately determined. Its effect on $HCO_3^-$ transport was measured in 11 ouabain-treated bladders bathed by $HCO_3^-$-rich, $Cl^-$-free ($Cl^-$ replaced by $SO_4^{2-}$) media in which $I_{sc}$ approximates the net reabsorption of $HCO_3^-$ [7, 14, 18]. In the presence of 1 mM vanadate, $I_{sc}$ (16.2 ± 2.2 $\mu$A) declined 76.1 ± 16.3% ($n$=5) after 30 min and approached near-zero values at about 60 min; with 0.1 mM vanadate present, $I_{sc}$ decreased 37.8 ± 9.2% ($n$=6) in 30 min and 47.9 ± 8.3% ($n$=6)

in 60 min. The inhibition of the $HCO_3^-$ moiety of $I_{sc}$ predicts a potent inhibition of mucosal acidification [7, 19] by vanadate.

One additional effect of serosal vanadate on the electrical parameters of ouabain-treated bladders is noteworthy. In about 50% of the bladders studied, vanadate caused two damped oscillations in the electrical parameters superimposed on the more slowly declining base-line values of these parameters as shown in Fig. 1 (A); of the remaining bladders, 35% exhibited one fluctuation in *PD*, $I_{sc}$, and *R*. Three or more fluctuations as shown in Fig. 1B were observed in three cases. These transients were observed under the following conditions: (i) 0.1 or 1 mM serosal vanadate; (ii) open-circuited or short-circuited bladders; (iii) $Cl^-$-rich or $Cl^-$-free media ($HCO_3^-$ present); and (iv) in the presence of either 0 or 1 mM vanadate in the mucosal fluid.

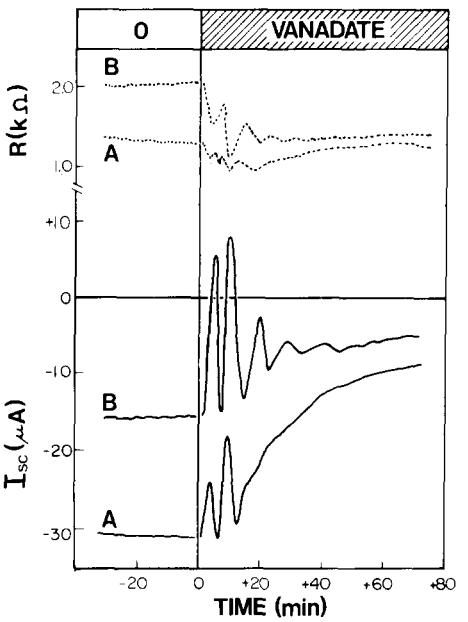


Fig. 1. Transient effect of vanadate on $I_{sc}$ and *R* of ouabain-treated bladders in $Cl^-$-rich and $Cl^-$-free bathing media. *R* is shown in upper panel (dotted curves); $I_{sc}$ is shown in lower panel (solid curves). Negative values of $I_{sc}$ indicate that serosa is electronegative to mucosa. The time course of *PD* has been omitted for clarity but can be estimated from $I_{sc} \times R$. Data from two experiments (A and B) are shown. Experimental conditions: (A) 1 mM vanadate; bathing medium described in legend to Table II; (B) 0.1 mM vanadate; bathing medium as described in legend to Table II, except that $Cl^-$ was replaced by $SO_4^{2-}$ without changing the concentration of cations. Osmolality was readjusted with sucrose.

Whereas the rapid fall in *PD*, $I_{sc}$, and *R* beginning 2 min after serosal vanadate addition may be caused, at least in part, by the onset of $H_2VO_4^-$ diffusion across the basolateral membrane, the subsequent transients in the electrical parameters are not readily explained by such a process. Oscillations in ionic fluxes have been observed in mitochondria isolated from other tissues [20]. However, unlike the harmonic oscillations induced by various weak acidic anions in mitochondria, the oscillations induced by vanadate in the turtle bladder have a longer and progressively increasing period (from 4 to 12 min over a total time of about 50 min in Fig. 1). A tentative hypothe-

sis is that a vanadate-induced hyperpolarization of the basolateral membrane, in part, causes changes in the conductive flows of ions other than vanadate across this membrane.

It is concluded that vanadate inhibits $Na^+$, $Cl^-$, and $HCO_3^-$ reabsorption in the turtle bladder with a relative potency of $HCO_3^- > Cl^- \sim Na^+$ transport. These data constitute the first demonstration of an inhibitory effect by vanadate on ouabain-insensitive epithelial anion transport. Earlier studies have shown that anion transport in the turtle bladder is not only ouabain-insensitive, but also $Na^+$-independent [9, 15]. Consequently, inhibition of electrolyte transport by vanadate is not restricted to a primary inhibition of cation transport, as has been frequently assumed. The potency of vanadate in turtle bladders is about 1/10 that of the disulfonic stilbenes in inhibiting $Cl^-$ and $HCO_3^-$ reabsorption [6, 7] and about 1/1000 that of oubain in inhibiting $Na^+$ transport [15].

To account for the observed vanadate-induced inhibition of anion and $Na^+$ transport the following is proposed. (i) Vanadate interacts with the anion-selective pathways (or carriers) in the basolateral membrane [6—8] to retard anion transport. Several factors might be involved, e.g., electrostatic repulsion and steric hindrance due to the complex structural and binding properties of this transition metal anion [5, 10, 21], although vanadate actions at other cellular sites [22, 23], including mitochondria, cannot be excluded. (ii) Upon penetrating via the anion paths, vanadate inhibits $Na^+$ transport by binding to the $(Na^+ + K^+)$-ATPase [15] on the cytoplasmic side of the membrane in a manner analogous to that described by Cantley et al. for red cells [5].

The concentration of vanadate used in this study was much higher than that used by Balfour et al. [1] in vivo. Nevertheless, the results are consistent with the notion that a vanadate-induced inhibition of active anion transport in the distal nephron segments [24] contributes to the potent diuretic action of this substance [1].

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