J. Membrane Biol. 88, 25–31 (1985)

The Journal of
Membrane Biology

Effects of the Anion Transport Inhibitor, SITS, on the Proximal Straight Tubule of the Rabbit Perfused *in vitro*

Bruce A. Biagi
Department of Physiology, Ohio State University, Columbus, Ohio 43210

Summary. Conventional microelectrodes were used to study the effects of SITS (4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonate) on the basolateral membrane potential *Vbl* of the superficial proximal straight tubule (PST) of the rabbit kidney perfused *in vitro*. Addition of 0.1 mM SITS to the bathing solution resulted in a slow and irreversible hyperpolarization of *Vbl* from −42.5 ± 1.17 (37) mV to −77.3 ± 0.83 (52) mV. The new steady-state potential was reached in 10 to 15 min and was accompanied by visible cell swelling. Associated with this *Vbl* hyperpolarization was: 1) an increased steady-state depolarization (from 6.2 ± 0.77 (17) mV to 25.7 ± 0.83 (29) mV) in response to increasing bath potassium concentration from 5 to 16.7 mM (HK); 2) a decreased transient depolarization (from 19.8 ± 1.88 (8) mV to 0.43 ± 0.37 (8) mV) in response to decreasing bath bicarbonate concentration from 22 to 6.6 mM at constant bath pH (L-HCO_3); and 3) inhibition of a depolarizing overshoot and a decreased steady-state depolarization (from 35.9 ± 1.84 (12) mV to 4.7 ± 1.37 (13) mV) in response to reducing bath sodium concentration from 144 to zero (0-Na). Sodium, chloride and NMDG (N-methyl-D-glucamine) were used as the substituting ions, respectively. These results are consistent with the presence of a coupled sodium-bicarbonate carrier in the basolateral membrane which is electrogenic and SITS inhibitable. Comparison of the time course of SITS effects on these ion-substitution responses suggests that the inhibition of the bicarbonate exit pathway(s) is the primary event and that the changes in *Vbl* and in the steady-state *Vbl* responses to HK and 0-Na are secondary events which may be related to changes in intracellular composition and/or basolateral membrane properties.

Key Words basolateral membrane potential · proximal straight tubule · SITS · potassium · sodium · bicarbonate

Introduction

The stilbene disulfonates SITS (4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid) and DIDS (4,4′-diisothiocyanostilbene-2,2′-disulfonic acid) have been used extensively to characterize anion exchange in the red blood cell membrane [10]. In systems other than red blood cells, inhibition of transport by SITS or DIDS is usually taken as evidence for the involvement of anion transport in the process. In addition to, and perhaps resulting from, the inhibition of chloride and bicarbonate transport, these inhibitors also affect the regulation of intracellular pH [21] and volume [17] in a variety of cells, and this is frequently linked to the transport of sodium ions in an electrogenic or charge-carrying manner [15, 17, 19].

Bicarbonate transport in the proximal tubule involves both luminal sodium-hydrogen exchange and the exit of bicarbonate across the basolateral membrane. Several pathways for bicarbonate exit have been proposed which appear to be highly species specific. In the rat proximal tubule perfused *in vivo* bicarbonate exit is conductive and is considered to be in the form of CO_2 and hydroxyl ion [8, 9, 25]. Burckhardt et al. [8] have shown that this pathway is not associated with the movement of sodium ions and is "instantaneously" blocked by 0.1 mM SITS in the peritubular perfusion solution. Peritubular SITS has also been shown to inhibit net bicarbonate reabsorption in the rat proximal tubule [13].

In the amphibian proximal tubule, bicarbonate exit is linked to the movement of sodium. Boron and Boulpaep [7] have characterized a 2 HCO_3 : 1 Na electrogenic transporter in *Ambystoma* proximal tubule. Guggino et al. [15] demonstrated the presence of sodium-linked chloride-bicarbonate exchange in the basolateral membrane of *Necturus* proximal tubule which may also be associated with charge movement. Matsumura et al. [19] have, in addition, demonstrated a conductive bicarbonate pathway in parallel with this system in the *Necturus*. These systems are also blocked by SITS.

In the rabbit proximal tubule perfused *in vitro* the mechanism for bicarbonate exit remains uncertain. Initial reports were unable to demonstrate either a significant bicarbonate conductance [2, 3, 6] or a sodium dependence of the basolateral membrane potential [2]. With improved techniques of bath exchange, however, it has been possible to

Table 1. Steady-state values of *Vbl* and the *Vbl* responses (ΔVbl) to ion substitutions under control conditions and following addition of 0.1 mM ISTS to the bathing solution

	Control	SITS
Vbl (mV)	−42.5 ± 1.17 (37)[a]	−77.3 ± 0.83 (52)
ΔVbl:		
L-HCO_3 (mV)	19.8 ± 1.88 (8)	0.4 ± 0.37 (8)
0-Na (mV)	35.9 ± 1.84 (12)	4.7 ± 1.37 (12)
HK (mV)	6.2 ± 0.77 (17)	25.7 ± 0.67 (17)

[a] Values are mean ± SE (number of cells).

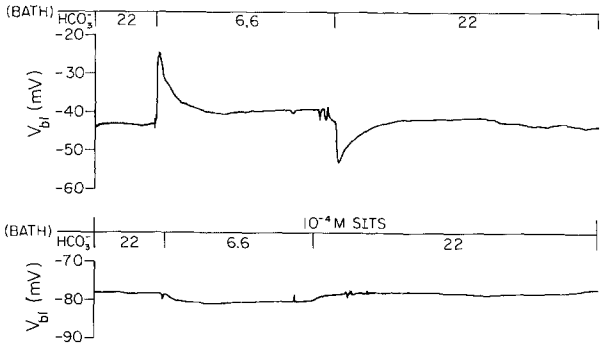

Fig. 1. The responses of the basolateral membrane potential *Vbl* to a decrease in bicarbonate concentration at constant bath pH of 7.4. Both tracings were obtained from the same PST cell. The upper panel shows a typical "control" response and the lower panel the absence of a response after 0.1 mM SITS had been added to the bath and a new steady-state *Vbl* had been obtained. Note the hyperpolarization of *Vbl* from −43 to −78 mV

provide evidence for both a conductive bicarbonate exit and a sodium-dependent pathway which is SITS sensitive [5].

It was the purpose of these studies to examine in greater detail the effects of SITS on bicarbonate transport in the rabbit proximal straight tubule perfused *in vitro*. The results show that SITS rapidly blocks both the conductive and sodium-dependent pathways. Secondary to this blockage is a marked hyperpolarization of the basolateral membrane potential which can be associated with an increased potassium selectivity of this membrane. In the steady state, these effects are accompanied by marked cell swelling.

Materials and Methods

The techniques for isolation and perfusion of individual nephrons from rabbit kidneys were similar to those used previously for the measurement of basolateral membrane potentials and potassium activities in the proximal straight and convoluted segments [3–6].

The basic "control" solution contained, in mM/liter: NaCl 118, KCl 5, $NaHCO_3$ 22, $MgSO_4$ 1.0, $CaCl_2$ 1.8, NaH_2PO_4 2.0, Na-acetate 2.0, glucose 8.3, and alanine 5.0. When equilibrated with a gas mixture of approximately 5% CO_2/95% O_2, this solution had a pH of 7.3 to 7.4. The osmolality was in the range of 290 to 295 mOsm/liter and all experimental solutions were adjusted to be equal to the measured value of the control solution.

Ion substitutions were made in the control solution in order to decrease bicarbonate (L-HCO_3), increase potassium (HK), and reduce sodium concentrations (0-Na). Bicarbonate concentration was reduced to 6.6 mM by replacement with Cl. A gas mixture with approximately 1% CO_2/99% O_2 was used to maintain constant pH. Potassium concentration was increased to 16.7 mM by substituting for sodium.

Solutions with reduced sodium concentration were made by totally replacing sodium with N-methyl-D-glucamine (NMDG) and a component of choline. The NMDG stock solution was titrated with HCl to a pH of 7.4 before mixing the test solution. The zero-sodium solution contained, in mM/liter: NMDG 118, KCl 5, choline HCO_3 22, $MgSO_4$ 1, KH_2PO_4 2, $CaCl_2$ 1.8, K acetate 2, alanine 5, glucose 8.3.

The luminal perfusate consisted of a low bicarbonate solution containing 6.7 mM HCO_3 and, in addition, glucose and alanine were replaced with 13.3 mM mannitol. This solution resembles that found in the late proximal tubule *in vivo*.

SITS was obtained either from Pierce Chemical Co., Rockford, Ill. or from ICN Biochemical, Cleveland, Ohio, and was added to the bath solutions in a concentration of 0.1 mM. In general, a new steady state in *Vbl* and in the responses to *Vbl* to the experimental solutions was reached approximately 10 min after exposure to SITS. In those experiments where the time course of SITS effects was examined, SITS was first added to the experimental solution to examine the instantaneous response. Subsequent control and experimental solutions contained SITS. In one series of experiments 0.1 mM DIDS was used to compare the effects on *Vbl*.

The basolateral membrane potential *Vbl* was measured using single-barreled microelectrodes. They were pulled from thick wall capillary tubing containing an internal fiber (Frederick Haer, o.d. = 1.2 mm). Electrodes were filled with 1.0 M KCl at the time of use and had a resistance in the range of 80 to 150 MΩ. A thin chlorided silver wire was inserted into the back of the microelectrode and used to connect the electrode to the input of a high-impedance electrometer (FD 223, W-P Instruments, New Haven, Conn.). The output from the electrometer was displayed and stored on a digital oscilloscope (Nicolet Instrument Corporation, Madison, Wis.) and tracings of the original responses were made on an X-Y recorder (Hewlett-Packard, Model 7035B).

Bath solution pH, Cl, K or Na were monitored depending upon the principle ion substitution being examined. Miniature ion-selective electrodes were constructed using ion-exchange resins and the methods used for K-selective microelectrode construction [6]. These had a tip diameter of approximately 40 μm and were positioned 50 to 100 μm from the cell under study. These electrode systems were used to quantitate the speed of solution exchange during each experiment. An exchange time of one second or less was accepted as satisfactory [4, 5].

Female New Zealand White rabbits were sacrificed by a blow to the head, and proximal straight tubules were dissected from the superficial cortex in a chilled bath of control solution and 10% rabbit serum. The initial values of *Vbl* were measured with control solution in the bath. Changes in *Vbl* (ΔVbl) were referenced to the control value where a positive value of ΔVbl indicates a depolarizing response as the intracellular potential becomes more positive relative to the bathing solution.

As mentioned previously, 10 to 15 min were required to achieve a new steady-state condition after SITS addition. Since the changes in any of the examined parameters were not reversible following 1 to 1.5 hr of perfusion with the control solution, the effects of SITS are essentially irreversible. For this reason, ion-substitution responses were measured in two to three cells under control conditions before SITS addition. True paired differences thus could only be made in one cell per tubule. A total of 65 cells were studied from 29 proximal straight tubule segments.

The results are expressed as means ± SE with the number of cells in parentheses. Statistical significance was determined using the Student's *t*-test for mean differences compared with zero. Statistical significance was accepted at $P < 0.05$.

Results

Steady-State Effects of SITS

A summary of the steady-state effects of 0.1 mM SITS in the bath solution is presented in Table 1 and examples of the transient responses to ion substitutions before and after SITS are illustrated in Figs. 1 and 2. The characteristic *Vbl* responses to the ion substitutions before SITS are shown in the upper panels of Figs. 1 and 2, and have been described previously [5]. Reducing bath bicarbonate concentration produced a transient spike depolarization with a peak value of 19.8 mV and a steady-state value within 5 mV of the control value. The zero-sodium solution also resulted in a transient depolarization but with a considerably larger steady-state depolarization (35.9 mV). The mean difference between the peak and steady-state values in the zero-sodium experiments was 7.3 ± 0.99 (12) mV. Increasing bath potassium resulted in a step depolarization of 6.2 mV.

In the steady-state conditions following addition of SITS to the bath, *Vbl* was significantly hyperpolarized (−42.5 *vs.* −77.3 mV). The bicarbonate transient was reduced to a value not different from zero. The zero-sodium response no longer included an initial peak and the steady-state depolarization was significantly reduced from 35.9 to 4.7 mV. In contrast, the depolarizing response to potassium was significantly increased from 6.2 to 25.7 mV.

In another short series of experiments involving two PST, 0.1 mM DIDS was added to the bathing solution. A similar hyperpolarization of *Vbl* from −44 ± 6.9 (4) mV to −70 ± 4.3 (5) mV was seen, and this was also irreversible.

Time Course of SITS Effects

The above results are consistent with the inhibition of both a bicarbonate conductance and a sodium-dependent bicarbonate pathway. An underlying question in the present experiments is whether the ion-substitution responses do indeed reflect two pathways in parallel, or perhaps the effects of inhibition of a single mechanism.

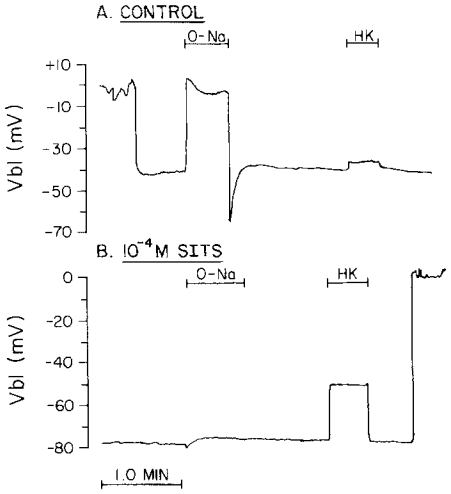

Fig. 2. Tracings taken from the same PST cell before and after SITS addition to the bath. The upper panel illustrates the initial puncture, and typical zero-sodium (0-Na) and high-K (HK) responses under control conditions. The lower panel illustrates these responses after SITS and also shows the withdrawal of the microelectrode back into the bath solution

Burckhardt et al. [8] have described the SITS inhibition of bicarbonate buffer conductance in the rat proximal tubule as "instantaneous." It seemed possible, therefore, that a close examination of the time course of SITS effects might provide information relevant to distinguishing primary and secondary effects of the inhibitor.

In these experiments, 0.1 mM SITS was first added to the ion-substituted solution to evaluate a possible instantaneous response, and the various ion substitution responses were examined at different times after the initial application of SITS. The results of these experiments are summarized in Figs. 3 to 5 and in Table 2.

Inhibition of the Bicarbonate Response

Figure 3 illustrates the changes in the bicarbonate response at several times between the control and final steady-state response at 11.5 min. In practice, 15 to 30 sec are required to execute a solution exchange sequence, but for clarity, only those tracings are shown which illustrate the progression of the changes.

Several points should be noted. First, the initial spike depolarization is rapidly inhibited. In fact, the height of the peak in the first trial (instantaneous

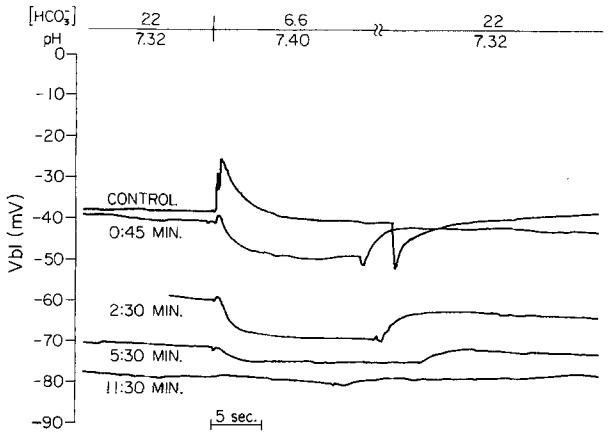

Fig. 3. Tracings taken from the same PST cell illustrating the time course of the SITS blockage of the low bicarbonate response. Tracings were taken 45 sec, 2.5, 5.5 and 11.5 min after addition of 0.1 mM SITS to the bathing solutions. Each trace was adjusted on the time axis so that the times for the low bicarbonate solution exchanges all coincided. Each trace is positioned to reflect the true value of *Vbl* at each time point

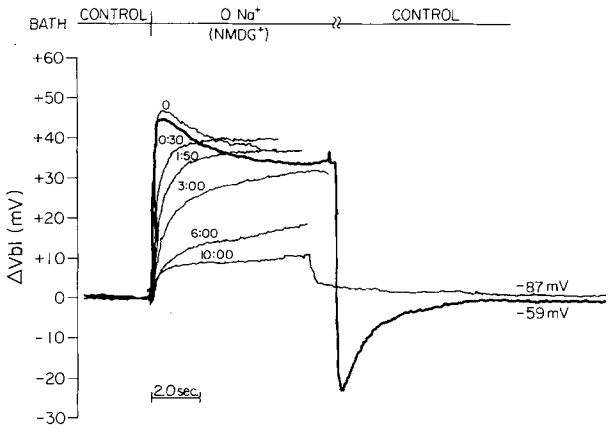

Fig. 4. Tracings taken from the same PST cell illustrating the time course of the inhibition of the zero-sodium response by 0.1 mM SITS added to the bathing solutions. The control response is drawn as a bold line and the control *Vbl* was −59 mV. The responses at the addition of SITS (0), and 30 sec, 1 min 50 sec, and 3, 6 and 10 min after SITS addition are superimposed on the same baseline to show the change in the *Vbl* response (ΔVbl) with time. The steady-state *Vbl* after SITS was −89 mV

response) was 80% of that measured before SITS addition. Second, at a time when the peak transient is almost completely inhibited at 45 sec *Vbl* has hyperpolarized very little; and, what appears to be the repolarization phase of the spike response is still present and is now seen as a hyperpolarizing response relative to the baseline value of *Vbl*. Third, this hyperpolarizing response decreases and finally disappears as the *Vbl* itself hyperpolarizes to the final steady-state value of −79 mV.

Inhibition of the Zero-Sodium Response

In Fig. 4 several zero-sodium responses have been superimposed on the same baseline in order to better illustrate the change in the shape of this response after SITS. The control response, represented by the bold line, had a control *Vbl* of −59 mV and the final trace at 10 min had a *Vbl* of −87 mV. Several points should again be noted. First, the initial peak, or overshoot, is rapidly inhibited. At 30 sec, in this case, it has completely disappeared. Second, a significant depolarizing response remains for several minutes. This gradually decreases until a 10-mV depolarization remains after 10 min of SITS exposure.

Increase in Potassium Response

The change in the high-K response is straightforward. As illustrated in Fig. 5, a small 2 to 3 mV response in this cell at a control *Vbl* of −39 mV becomes a 20-mV response at a final *Vbl* of −78 mV after 11.5 min of SITS exposure. There appeared to be no other consistent changes in the pattern of this response in any of the cells studied.

In order to quantitate the differences in the time responses to SITS, the observed changes were plotted as a percent of the maximal response *vs.* time for each of the cells examined. The time required to reach 50% of the final response was interpolated and are compared in Table 2. In comparing these values it is clear that the inhibition of the bicarbonate spike and of the zero-sodium overshoot are significantly faster than either the high-K, zero-sodium steady-state depolarization, or the hyperpolarization of *Vbl*. The times for each of these latter changes were not significantly different from each other. In general, then, two time scales can be described: an initial inhibition of the bicarbonate spike and zero-sodium overshoot which are 50% complete in approximately 30 sec; and a slower sequence of events which occur on a time scale of approximately 5 min.

Finally, it should be noted that SITS also resulted in marked cell swelling. This effect was not quantitated, but was clearly visible in the microscope. Cells became rounded, lost definition of the brush border, and had a granular appearance. Surprisingly, these cells maintained stable intracellular potentials of approximately −80 mV, and cells from a given tubule could be sampled with *Vbl*'s of this magnitude for 45 to 60 min after SITS addition.

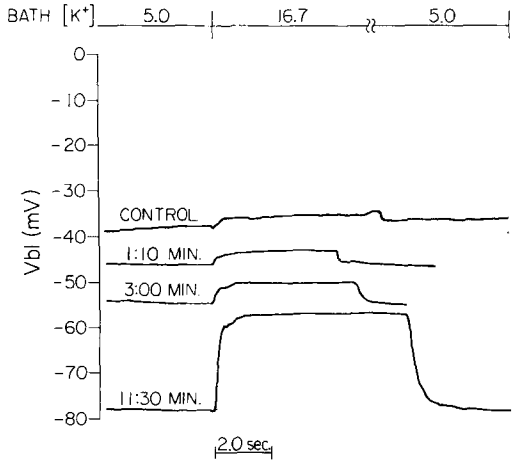

Fig. 5. Tracings taken from the same PST cell illustrating the time course of the SITS enhancement of the high-K response. Each trace was positioned as in Fig. 3 and the times after SITS addition are indicated. The final steady-state *Vbl* and HK response are illustrated by the 11 min 30 sec trace

Discussion

The results of these experiments demonstrate that basolateral SITS produces profound changes in the characteristics of the proximal straight tubule perfused *in vitro*. There is the strong indication that these involve both primary and secondary effects on this membrane border and on the intracellular volume and ion composition.

The interpretations placed upon the normal *Vbl* responses to the ion substitutions are critical to the evaluation of the SITS effects. As discussed previously [5], the lack of intracellular activity data, particularly with regard to sodium and pH, requires a fundamental assumption. This is that the *Vbl* responses to bicarbonate and sodium, because of their similarity to responses in other systems, reflect the transport pathways for bicarbonate which have been described in the rat [8, 9, 25] and amphibian [7, 15, 19] proximal tubules. Thus, it is assumed that the bicarbonate spike reflects a simple bicarbonate conductance and that the zero-sodium response reflects a sodium-dependent bicarbonate pathway. This parallel arrangement is similar to that described by Matsumura et al. [19] for *Necturus* proximal tubule.

Primary Effects of SITS

The results of the time-course studies indicate that the primary event is the inhibition of conductive and sodium-dependent bicarbonate exit pathways. This is evidenced by the rapid inhibition of the bicarbonate spike and the initial peak of the zero-sodium responses. Because electrophysiological methods have been used, it is not possible to evaluate changes in electroneutral transport systems, principally of Cl-HCO_3 exchange. Recent evidence, however, indicates that Cl-HCO_3 exchange is not a major pathway for bicarbonate movement across this membrane [20]. The fact that *Vbl* is only slightly hyperpolarized at a time when these pathways are blocked indicates that the SITS-sensitive pathways, as defined by the initial effects of SITS, normally contribute little to the resting potential in these cells.

Table 2. Time to reach 50% of the SITS response

Responses	Time (min)
Transients:	
HCO_3 spike	0.4 ± 0.05 (4)[a]
zero-Na overshoot	0.6 ± 0.22 (4)
Steady state:	
zero-Na depolarization	6.1 ± 1.11 (4)
high-K depolarization	4.8 ± 1.68 (4)
Vbl hyperpolarization	4.0 ± 0.60 (13)

[a] Values are mean ± SE (number of tubules).

The relatively small immediate effect of SITS on the zero-sodium response suggests that the principle depolarization seen 15 to 30 sec after bath sodium is reduced, may not, in fact, be associated with anion transport. A slower inhibition of some facet of the transporter mechanism is, of course, a possibility, and other alternate hypotheses are discussed below. It is clear, however, that SITS does influence the initial portion of this response and that this is consistent with rapid inhibition of a coupled sodium-bicarbonate exit pathway.

Since both the bicarbonate spike and the zero-sodium overshoot were rapidly inhibited by SITS, the data do not allow a distinction to be made between the presence of two parallel pathways (conductive and Na dependent) and a single sodium-dependent pathway for bicarbonate exit. While there is considerable evidence for a bicarbonate conductance in the rat proximal convoluted tubule [8, 9, 25], Alpern [1], using direct measurements of intracellular pH, has recently demonstrated that a major portion of peritubular H/OH/HCO_3 transport is linked to sodium in this segment. Our data are, in fact, also consistent with the hypothesis that a single Na-dependent pathway facilitates bicarbonate exit in the rabbit proximal straight tubule. The observation that *Vbl* changes little when the bicarbonate spike is completely inhibited would argue against a large bicarbonate conductance in this

membrane. Thus, the bicarbonate spike and initial zero-Na depolarization may simply reflect a single Na-dependent transport pathway. A simple bicarbonate-conductive component cannot be ruled out, however, and until this can be done, it will be assumed that two parallel pathways are present.

Secondary Effects of SITS

The secondary SITS effects which can be reasonably explained are the hyperpolarization of *Vbl* and the increased response to high-K solution. Clearly these represent the same effect, namely, an increased relative potassium selectivity of the basolateral membrane. The average HK response of 25.7 mV can be compared with the theoretical maximum change of 32 mV which is calculated using the Nernst equation and assuming that intracellular potassium concentration does not change. Considering the speed of the response (Fig. 5) and the fact that the change in extracellular potassium was small, this assumption seems valid. No information is available regarding the relative membrane and shunt resistances under these conditions, but the presence of a shunt resistance would be expected to reduce the observed *Vbl* response. Thus, it would appear that the basolateral membrane approaches a pure potassium electrode in the new steady state following SITS application.

Several hypotheses might explain the increased potassium selectivity of the basolateral membrane. The first is that the block of bicarbonate exit pathways causes an intracellular alkalinization and that this results in an increased potassium selectivity. Results from this laboratory have shown a considerable dependence of *Vbl* on the pH of the bathing solution [3, 4]. Decreasing bath pH decreases potassium permeability and produces a depolarization of approximately 35 mV/pH unit. If there is a similar dependence of internal pH, then an increase in K selectivity would be expected and would be consistent with the observed *Vbl* hyperpolarization and increased HK response.

The hyperpolarizing response to reduced bicarbonate concentration observed during the transition to the SITS steady state (Fig. 3) may provide support for the above interpretation. The repolarization phase of the control response has been interpreted to represent the loss of intracellular bicarbonate following the reduction in the bath concentration [9, 21]. With bicarbonate exit blocked, the decrease in bath CO_2 may now play a more critical role. If intracellular CO_2 decreases without a change in intracellular bicarbonate concentration, an intracellular alkalinization, increased K permeability, and *Vbl* hyperpolarization could result. As the membrane becomes more K selective and approaches *Ek* (the Nernst equilibrium potential across the basolateral membrane), changes in K selectivity would have less of an effect on *Vbl*. This agrees with the disappearance of this response as *Vbl* hyperpolarizes and the HK response increases. Clearly, a direct evaluation of the potassium gradient across the basolateral membrane following SITS will be of interest. Previous estimates of *Ek* under control conditions gave a value of −68 mV [6] which, when compared to a *Vbl* of −77 mV, suggests that intracellular K may increase secondary to SITS.

It should be noted that, in the above discussion, an increase in relative K selectivity of the basolateral membrane could result from either a direct increase in potassium permeability or from a decrease in the permeability to another ion whose equilibrium potential is positive relative to *Ek*. Either or both of these possibilities would result in an increased relative potassium permeability of the basolateral membrane. The relatively slow hyperpolarization of *Vbl*, however, argues that the block of a major conductive, depolarizing pathway is not a primary effect of the inhibitor. A direct evaluation of membrane conductances may provide a means to evaluate these possibilities [12].

A second hypothesis to account for an increased K selectivity of the basolateral membrane involves interaction with intracellular calcium regulation. It has been shown that DIDS can inhibit the Ca^{++}-ATPase of the red cell membrane [11], and there is evidence for a similar calcium pump in the basolateral membranes of both rat [14, 22] and rabbit [18] proximal tubules. If SITS inhibits the calcium pump, a rise in cell calcium might result and produce an increase in K permeability. Calcium dependence of potassium transport has been demonstrated in a variety of systems [24].

Finally, the observation that the cells swell following SITS suggests a third hypothesis: that cell swelling increases potassium permeability. An increase in net K and Cl loss is a common finding for the volume regulatory decrease in osmotically enlarged cells [18]. A similar increase in K permeability may be induced by cell swelling following SITS.

The nature of the zero-sodium depolarization which is not immediately inhibited by SITS remains unclear. A slow inhibition resulting from a low-affinity interaction with an electrogenic sodium-bicarbonate coupled system is a possibility. As discussed previously [5], several other interactions at the basolateral membrane may also be possible. These include: 1) an inhibition of the Na-K pump resulting from decreased intracellular sodium activity [12]; 2) a passive permeability to the major substituting cat-

ion, NMDG; 3) an interaction with the organic anion and/or organic cation transport systems of the proximal tubule [16, 23]; and 4) an effect on electrogenic Na^+–Ca^{++} exchange in this membrane [14]. Any of these alternate mechanisms may, in fact, play a role in the observed zero-sodium response.

Further clarification, however, of the zero-sodium response, as well as support for the conclusions drawn above, will require intracellular activity measurements of the ions involved. The requirement for fast time resolution in such measurements is underscored by the speed of the ion-substitution responses of *Vbl* in these cells. Recent advances in double-barreled microelectrode fabrication indicate that such measurements may be possible [25].

The author wishes to thank Ms. Barbara Brown for her excellent technical and editorial assistance. This work was supported by Public Health Service Grant AM-30694.

References

1. Alpern, R.J. 1985. Mechanism of basolateral membrane bicarbonate transport in the rat proximal convoluted tubule (PCT). *Clin. Res.* **33:**475A
2. Bello-Reuss, E. 1982. Electrical properties of the basolateral membrane of the straight portion of the rabbit proximal renal tubule. *J. Physiol. (London)* **326:**49–63
3. Biagi, B.A., Kubota, T., Sohtell, M., Giebisch, G. 1981. Intracellular potentials in rabbit proximal tubules perfused in vitro. *Am. J. Physiol.* **240:**F200–F210
4. Biagi, B.A., Sohtell, M. 1985*a*. pH sensitivity of the basolateral membrane of the rabbit proximal tubule. *Am. J. Physiol. (in press)*
5. Biagi, B.A., Sohtell, M. 1985*b*. Basolateral bicarbonate transport in the rabbit proximal tubule: Electrophysiological evidence for parallel pathways. *Am. J. Physiol. (in press)*
6. Biagi, B.A., Sohtell, M., Giebisch, G. 1981. Intracellular potassium activity in the proximal straight tubule. *Am. J. Physiol.* **241:**F677–F686
7. Boron, W.F., Boulpaep, E.L. 1983. Intracellular pH regulation on the renal proximal tubule of the salamander. Basolateral HCO_3 transport. *J. Gen. Physiol.* **81:**53–94
8. Burckhardt, B.-C., Cassola, A.C., Frömter, E. 1984. Electrophysiological analysis of bicarbonate permeation across the peritubular cell membrane of rat kidney proximal tubule. II. Exclusion of HCO_3 effects on other ion permeabilities and of coupled electroneutral HCO_3 transport. *Pfluegers Arch.* **401:**43–51
9. Burckhart, B.-C., Sato, K., Frömter, E. 1984. Electrophysiological analysis of bicarbonate permeation across the peritubular cell membrane of rat kidney proximal tubule. I. Basic observations. *Pfluegers Arch.* **401:**34–42
10. Cabantchik, Z.I., Knauf, P.A., Rothstein, A. 1978. The anion transport system of the red blood cell. The role of membrane protein evaluated by the use of 'probes.' *Biochim. Biophys. Acta* **515:**239–302
11. Carafoli, E., Zurini, M. 1982. The Ca^{2+}-pumping ATPase of plasma membranes. Purification, reconstitution and properties. *Biochim. Biophys. Acta* **683:**279–301
12. Cardinal, J., Lapointe, J.-Y., Laprade, R. 1984. Luminal and peritubular ionic substitutions and intracellular potential of the rabbit proximal convoluted tubule. *Am. J. Physiol.* **247:**F352–F364
13. Chan, Y.L., Biagi, B.A., Giebisch, G. 1982. Control mechanisms of bicarbonate transport across the rat proximal convoluted tubule. *Am. J. Physiol.* **242:**F532–F543
14. Gmaj, P., Murer, H., Kinne, R. 1979. Calcium ion transport across plasma membranes isolated from rat kidney cortex. *Biochem. J.* **178:**549–557
15. Guggino, W.B., London, R., Boulpaep, E.L., Giebisch, G. 1983. Chloride transport across the basolateral cell membrane of the *Necturus* proximal tubule: Dependence on bicarbonate and sodium. *J. Membrane Biol.* **71:**227–240
16. Hong, S.K., Goldinger, J.M., Song, Y.K., Koschier, F.J., Lee Sang Ho. 1978. Effect of SITS on organic anion transport in the rabbit kidney cortical slice. *Am. J. Physiol.* **234:**F302–F307
17. Kregenow, F.M. 1981. Osmoregulatory salt transporting mechanisms: Control of cell volume in anisotonic media. *Annu. Rev. Physiol.* **43:**493–505
18. Mandel, L.J., Murphy, E. 1984. Regulation of cytosolic free calcium in rabbit proximal renal tubules. *J. Biol. Chem.* **259:**11188–11196
19. Matsumura, Y., Cohen, B., Guggino, W.B., Giebisch, G. 1984. Electrical effects of potassium and bicarbonate on proximal tubule cells of *Necturus*. *J. Membrane Biol.* **79:**145–152
20. Nakhoul, N.L., Boron, W.F. 1985. Intracellular pH regulation in rabbit proximal straight tubules: Basolateral HCO_3 transport. *Kidney Int.* **27:**286 *(Abstr.)*
21. Roos, A., Boron, W.F. 1981. Intracellular pH. *Physiol. Rev.* **61:**296–434
22. Schonfeld, W., Menke, K.-H., Schonfeld, R., Repke, K.R.H. 1984. Evidence against parallel operation of sodium/calcium antiport and ATP-driven calcium transport in plasma membrane vesicles from kidney tubule cells. *Biochim. Biophys. Acta* **770:**183–194
23. Takano, M., Inui, K.-I., Okano, T., Saito, H., Hori, R. 1984. Carrier-mediated transport systems of tetraethylammonium in rat renal brush-border and basolateral membrane vesicles. *Biochim. Biophys. Acta* **773:**113–124
24. Yingst, D.R., Hoffman, J.F. 1984. Ca-induced K transport in human red blood cell ghosts containing arsenazo III: Transmembrane interactions of Na, K, and Ca and the relationship to the functioning Na-K pump. *J. Gen. Physiol.* **83:**19–45
25. Yoshitomi, K., Frömter, E. 1984. Cell pH of rat renal proximal tubule *in vivo* and the conductive nature of peritubular HCO_3(OH) exit. *Pfluegers Arch.* **402:**300–305

Received 9 May 1985; revised 16 July 1985