J. Membrane Biol. 12, 301 – 315 (1973)

Ouabain-Dependent Potassium-Potassium Exchange in the Toad Bladder

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Received 2 June 1972

Summary. Recent results from this laboratory have indicated the existence of two potassium compartments in the isolated toad bladder. Only one of these, containing less than 10% of total intracellular potassium, appears to be related to the sodium transport system, since potassium influx at the serosal border of this compartment is coupled to the sodium efflux which occurs there. Ouabain, which specifically inhibits serosal sodium exit, has no effect on potassium fluxes and compartment sizes in bladders mounted in normal (2.5 mM K) Ringer's solution. However, in the presence of this inhibitor, removal of serosal potassium results in a significant decrease in the rate coefficient for potassium efflux into the serosal medium, while an increase in serosal potassium results in a significant rise in this parameter, which appears to saturate at approximately 5 mM K. This sensitivity to serosal potassium is seen neither in the absence of ouabain nor when the sodium pump is inactivated by removal of sodium from the mucosal medium. Furosemide, which also inhibits the sodium transport system, both inhibits potassium transport parameters in normal Ringer's and abolishes the potassium-sensitive potassium efflux seen in the presence of ouabain. Thus, the Na–K pump appears to operate as a K–K exchanger when the sodium system is inhibited by ouabain; this K–K exchange mechanism is inhibited by furosemide. One explanation for these results is that ouabain effects an alteration in the affinities of the transport system for sodium and potassium.

Many epithelial membrane systems have been shown to carry out the net transport of sodium against an electrochemical potential gradient. Since the electrochemical potential of potassium inside these cells is generally considered to be greater than that in the medium, it has been long thought that the active transport system located at the inside border of these systems is a coupled Na–K exchange pump. However, because of the difficulty in measurement of Na and K fluxes at the individual borders of the cells, the details of these transport mechanisms have been difficult to determine.

We have recently reported that it is indeed possible to measure the kinetics of either sodium (Finn & Rockoff, 1971) or potassium (Finn & Nellans, 1972) movements at each of the two opposite-facing membranes

of the toad bladder epithelial layer, and have also shown that it is possible to determine the size of the transport pool for each of these two cations. Our data also suggested that the Na extrusion and K uptake mechanisms at the serosal border of these cells are indeed coupled, and furthermore that the coupling ratio cannot be distinguished from a value of 1. Our model showed that there were two K compartments contained within the toad bladder epithelium, only one of which appeared to share a mechanism with the Na system. In the experiments presented in the present paper, we have examined some further aspects of the K transport system.

Our data indicate the following: in the presence of ouabain, K–K exchange replaces the Na–K exchange normally present at the serosal border. This system is inhibited by furosemide, is not seen in the absence of ouabain, and requires the presence of sodium in the mucosal bathing medium. It will be suggested that ouabain alters the affinity of the Na efflux system, such that potassium competes effectively for sodium at that site.

Materials and Methods

The toads used in these studies were *Bufo marinus* of Colombian origin, and were obtained from the Pet Farm, Miami, Florida, or the Tarpon Zoo, Tarpon Springs, Florida. The toads were pithed, and the bladder was removed and placed in Ringer's solution, composed, in mM/liter, of NaCl 109, KCl 2.5, $CaCl_2$ 0.9, $NaHCO_3$ 2.4, and glucose 5.6. Solutions were gassed with room air and had a pH of about 7.8. The bladders were mounted between halves of a Lucite chamber which has been described previously (Finn & Rockoff, 1971), and were kept short-circuited throughout all experiments. ^{42}K at a final concentration of 2.5 mM (except as described below) was added to the serosal bathing medium, allowed to reach a steady state within the tissue, and then a washout experiment was performed as previously described (Finn & Nellans, 1972).

Briefly, the technique involves collecting effluent from each chamber separately at 30-sec intervals for 30 min, and fitting the count-rate data (corrected for decay) to sums of exponentials. From these fits, and from the utilization of standard methods of compartmental analysis, the K pools, fluxes, and rate coefficients may be determined.

In experiments in which sodium was removed from either or both media, it was replaced on a mole-for-mole basis by choline chloride (recrystallized from hot ethanol). The concentration of potassium in the bathing media was altered by substitution with sodium. Ouabain (G-strophanthin) or furosemide was added to the serosal medium in a final concentration of 10^{-3} M, and was present for at least 1 hr prior to the start of a washout study. At the end of each series of washout experiments on a given bladder, the bladder was punched out of the assembly with a cork borer, dried for at least 48 hr at 90 °C, and the dry weight obtained.

Results

Effect of Ouabain on Steady-State Potassium Fluxes

Since it is known that ouabain inhibits active Na transport in this preparation (Herrera, 1968), the effects of this inhibitor were tested on the K

Table 1. Effect of ouabain on potassium kinetics ($n=9$)

	A. Fast pool					
	J_{Na}^{Net}	J_{1M}	J_{1S}	A_1	k_{M1}	k_{S1}
Control	0.504 ±0.080	0.0126 ±0.0041	0.7153 ±0.1931	1.546 ±0.348	0.0084 ±0.0022	0.4501 ±0.0504
Ouabain, 10^{-3} M	0.102 ±0.037	0.0172 ±0.0072	1.0521 ±0.2741	2.139 ±0.416	0.0073 ±0.0026	0.4473 ±0.0648
$p_{difference}$ [a]	<0.01	NS	NS	NS	NS	NS
	B. Slow pool					
	J_{2M}	J_{2S}	A_2	k_{M2}	k_{S2}	
Control	0.0044 ±0.0014	0.2332 ±0.0566	5.915 ±1.286	0.0007 ±0.0001	0.0401 ±0.0036	
Ouabain, 10^{-3} M	0.0047 ±0.0014	0.1611 ±0.0359	5.394 ±1.050	0.0011 ±0.0003	0.0325 ±0.0041	
$p_{difference}$	NS	NS	NS	NS	NS	

[a] $p_{difference}$ is the probability that the difference between control and experimental (in this case, ouabain addition) values is equal to zero. J_{ij} is the flux (μEquiv × min^{-1} × 100 mg dry wt^{-1}) and k_{ij} the rate coefficient (min^{-1}) for movement into compartment i from compartment j. J_{Na}^{Net} is the net transepithelial Na flux (μEquiv × min^{-1} × 100 mg dry wt^{-1}), taken as equivalent to the short-circuit current. A_i is the compartment size, in μEquiv × 100 mg dry wt^{-1}. Since the total tissue potassium is in the steady state, and since there is no significant net transepithelial K flux, $J_{ij}=J_{ji}$. NS = not significant. The subscripts M and S refer to the mucosal and serosal media, and the subscripts 1 and 2 to the fast and slow tissue compartments. Values are given as means ± SEM.

This legend applies to all tables.

kinetics. In each of these studies a control washout was performed first, and then a second washout performed in the presence of ouabain. The results are shown in Table 1, where each of the kinetic parameters of the two measurable tissue K pools is shown as the mean of nine separate pairs of experiments. It should be noted that despite a mean decrease in net Na transport (as measured by the short-circuit current) of some 80%, there were no significant changes in any of the measured fluxes, rate coefficients, or pools. These ouabain results were surprising, since it was expected that there would be a diminution of the K influx at the serosal border, J_{1S}, especially since our previous data indicated that there is fairly tight Na–K coupling at this site. However, one explanation for these results might be that there is some exchange of intracellular for extracellular potassium in the presence of ouabain. To document this possibility, as well as to evaluate the correctness of the model, the following studies were done.

Effect of Changes in Serosal Potassium on Steady-State Potassium Kinetics

In this series of experiments, a control washout was performed as before. Subsequently (and in some cases this was done first), the K concentration of the serosal bathing medium was increased to 10 mM, both in the loading solution and in the wash solution. Under these circumstances, there is a transient fall in the short-circuit current, followed by a rise to a new steady value. The initial fall presumably reflects the movement of potassium into the cells as the gradient of potassium across the serosal border changes; the final steady state of short-circuit current, which is often higher than baseline, possibly reflects a stimulatory effect of potassium on the Na transport system (Sullivan, Tucker & Scherbenske, 1971). Studies to evaluate this further are currently under way. In each study, the tissue was allowed to remain in contact with the high K concentration for at least 90 min prior to the start of the washout, so that it could be reasonably safe to assume that total tissue potassium was in a steady state.

The results are shown in Table 2 and indicate a significant increment in both serosal influx of potassium and in the size of the K pool. There were no changes in any of the parameters in the slower compartment in this or in any other studies, so that these data are not shown. It is of particular interest that there were no significant changes in the rate coefficients for exit of potassium at either the mucosal or the serosal border. We shall return to this point later.

In two additional studies, three different K concentrations were tested. The results are shown in Table 3 and again show a rather consistent relationship between serosal K concentration, serosal entry rate, and pool size. Again, however, there is no consistent effect of these changes on the rate coefficients for exit of potassium.

Thus, the technique is sensitive enough to detect changes in kinetics when the K concentration in the medium is altered; we therefore would

Table 2. Effect of changes in serosal potassium on potassium kinetics in the steady state ($n=6$)

	J_{1M}	J_{1S}	A_1	k_{M1}	k_{S1}
Control washout, [K] = 2.5 mM at all times	0.0164 ±0.0063	0.3579 ±0.1366	2.592 ±1.345	0.0151 ±0.0063	0.2144 ±0.0943
Experimental washout, [K] = 10 mM at all times	0.0721 ±0.0263	1.307 ±0.381	6.649 ±1.711	0.0100 ±0.0011	0.2088 ±0.0458
$p_{\text{difference}}$	NS	<0.02	<0.01	NS	NS

Table 3. Results of two separate experiments with three different steady-state potassium concentrations

		J_{1M}	J_{1S}	A_1	k_{M1}	k_{S1}
Exp. 1	K = 1	0.0026	0.0495	0.225	0.0117	0.2195
	K = 2.5	0.0235	0.3880	2.470	0.0095	0.1570
	K = 10	0.0232	0.7242	5.156	0.0045	0.1404
Exp. 2	K = 1	0.0007	0.0190	0.103	0.0063	0.1847
	K = 2.5	0.0039	0.1668	0.856	0.0092	0.1949
	K = 10	0.0193	0.4582	2.182	0.0089	0.2099

In each experiment, both load and wash solutions contained potassium at the concentration indicated in column 1.

have expected changes after the addition of ouabain. The possibility remains, however, that in the presence of this inhibitor, there is a K–K exchange system at the serosal side which maintains the K pool. This was evaluated by determining the effect of changes in the external K concentration on K efflux.

Effect of Step Changes in Potassium Concentration on Potassium Efflux in the Presence of Ouabain

As we have previously shown (Finn & Nellans, 1972), the determination of K fluxes and pools depends upon the steady state for total potassium. On the other hand, we have also shown that the measurement of the rate coefficients for efflux (k_{S1} and k_{M1}) depends only on the steady state for tracer within the tissue at the start of the washout. In each of the studies to be described, then, it is possible, during the washout into a solution containing a concentration of the potassium different than that which was in the load solution, to measure only the rate coefficients for efflux. It should also be remembered that during the initial one minute of the washout, the rate of flow through the chamber is such that the half time for washout of the chamber is approximately 2.4 sec (Finn & Rockoff, 1971), so that during this one minute there is essentially a step change in the K concentration to whatever concentration is present in the washing solution. Furthermore, since no changes were seen in the rate coefficient for efflux from the slow compartment, all changes which we are observing occur in that component which has a half time of approximately 2 min (Finn & Nellans, 1972). Experiments were performed in which the washout solution contained 0, 5, 10 or 20 mM potassium. In each experiment, a control

Table 4. Effect of serosal potassium on potassium efflux in the presence of ouabain

$[K]_S$ during load	$[K]_S$ during wash	n		k_{M1}		k_{S1}
2.5	2.5	5		0.0235 ± 0.0012		0.2244 ± 0.0614
2.5	0			0.0204 ± 0.0016		0.1846 ± 0.0580
			$\Delta =$	0.0031 ± 0.0015	$\Delta =$	0.0398 ± 0.0106
				NS		$p < 0.02$
2.5	2.5	5		0.0202 ± 0.0025		0.1928 ± 0.0392
2.5	5			0.0305 ± 0.0056		0.2633 ± 0.0251
			$\Delta =$	−0.0103 ± 0.0047	$\Delta =$	−0.0705 ± 0.0182
				NS		$p < 0.02$
2.5	2.5	5		0.0322 ± 0.0359		0.2154 ± 0.0679
2.5	10			0.0359 ± 0.0125		0.2805 ± 0.0779
			$\Delta =$	−0.0037 ± 0.0142	$\Delta =$	−0.0651 ± 0.0106
				NS		$p < 0.01$
2.5	10	5		0.0559 ± 0.0237		0.3211 ± 0.0668
2.5	20			0.0363 ± 0.0103		0.3050 ± 0.0303
			$\Delta =$	0.0196 ± 0.0196	$\Delta =$	0.0160 ± 0.0240
				NS		NS

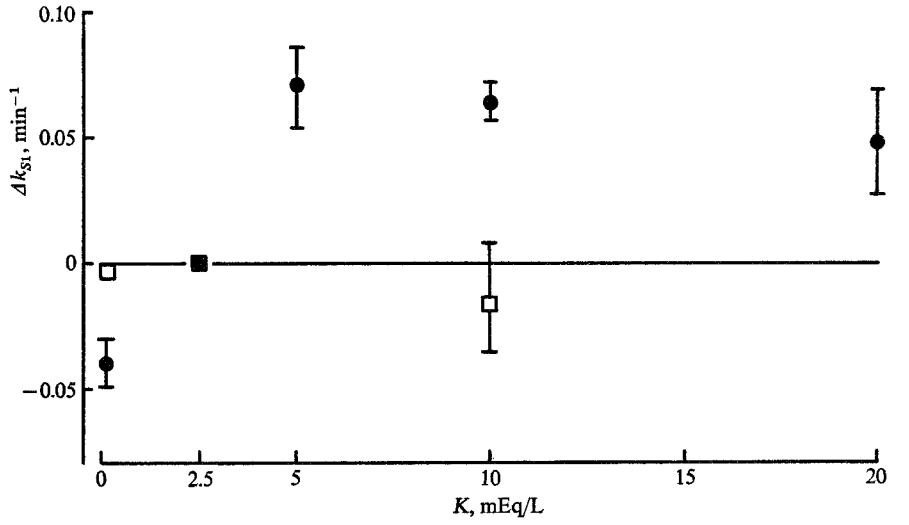

Fig. 1. Effect of external K on K efflux. Each point, with the exception of the one at 20 mM K, represents the mean change in the rate coefficient for K efflux k_{S1} when serosal potassium is changed at the start of the washout, from 2.5 mM to the concentration on the abscissa. The point at 20 mM K represents the changes in k_{S1} in those experiments in which the concentration was changed from 10 mM to 20 mM; for convenience, this point is plotted as though the point at 10 mM K were the baseline value. Closed circles are experiments with ouabain alone; ($n = 5$ at each point); open squares represent experiments performed in the presence of both ouabain and furosemide ($n = 1$ at 0 K, $n = 4$ at 10 K). Bars represent ± SEM

Table 5. Effect of furosemide on potassium kinetics

	A. Furosemide alone, steady-state experiments ($n=5$)				
	J_{1M}	J_{1S}	A_1	k_{M1}	k_{S1}
Control	0.0240 ±0.0093	0.6424 ±0.2430	1.070 ±0.300	0.0260 ±0.0135	0.5365 ±0.0628
Furosemide, 10^{-3} M	0.0306 ±0.0147	0.4059 ±0.1796	1.001 ±0.252	0.0296 ±0.0122	0.3488 ±0.0599
$p_{\text{difference}}$	NS	<0.025	NS	NS	<0.01
	B. Furosemide + ouabain, step changes in serosal potassium ($n=4$)				
Control [K] = 2.5 at all times				0.0127 ±0.0052	0.4443 ±0.1449
Experimental					
[K] = 2.5 during load				0.0272	0.4287
[K] = 10 during washout				±0.0251	±0.0849
$p_{\text{difference}}$				NS	NS

washout was performed (K = 2.5 mM) either before or after a study in which the washout solution was altered. The results are shown in Table 4. Note that a reduction in K concentration in the serosal solution results in a decrease in the rate of tracer movement into that medium, while an increase in serosal potassium results in a significant rise in the efflux rate coefficient of tracer into that medium. For convenience, the data are also plotted in Fig. 1, where the difference in the rate coefficient for efflux, k_{S1}, between the control and the experimental preparation is plotted against the serosal K concentration. It is clear that this K-sensitive K efflux seen in the presence of ouabain appears to saturate at a serosal K concentration near 5 mM. Note also that there is no effect of changes in serosal K concentration on the rate coefficient for efflux of potassium into the mucosal medium, as shown in Table 4.

Effects of Furosemide

Furosemide, another inhibitor of Na transport, also produced a significant decrease in the short-circuit current in these experiments when added to the serosal medium in a final concentration of 10^{-3} M. To evaluate the effects of this change on steady-state K kinetics, experiments were performed similar to those previously described for ouabain. The results of these

Table 6. Effect of ouabain and furosemide

	J_{1M}	J_{1S}	A_1	k_{M1}	k_{S1}
Ouabain 10^{-3} M [K] = 2.5 at all times	0.0409	0.2941	0.8654	0.0473	0.3399
Ouabain + furosemide [K] = 2.5 at all times	0.0420	0.1924	0.8464	0.0494	0.2274
Ouabain + furosemide [K] = 2.5 during load [K] = 0 during wash				0.0541	0.2247

studies are shown in Table 5A. Unlike the effects of ouabain, the addition of furosemide resulted in a significant decrease in the K influx at the serosal border, and also in a significant decrease in the rate coefficient for K efflux at the same site. On the other hand, there were no changes in any of the other parameters of this compartment, and as in other situations, no changes in any of the parameters of the slow K compartment. In view of this apparently different mode of action of furosemide, studies were performed in which the effect of furosemide on the ouabain-induced K-dependent K efflux shown above were tested. In these experiments both furosemide and ouabain were present in the serosal medium. The control washout was performed with a concentration of 2.5 mM potassium present at all times. The subsequent washout was done, after loading again at a concentration of 2.5 mM, into a solution containing 10 mM potassium. As shown in Table 5B, there was now no significant change in the K efflux rate coefficient following the step increase in K concentration in the medium. The results of a single set of experiments of this type are shown in Table 6, which indicates also that there was no effect of reducing the external concentration of potassium to zero during the wash when both furosemide and ouabain were present. Again, these results differ from those seen with ouabain, and the furosemide results are also plotted in Fig. 1.

Thus, furosemide not only inhibits both steady-state K entry and exit, but also inhibits the K-dependent K-efflux seen in the presence of ouabain.

Effect of Step Changes in Serosal Potassium on Potassium Efflux in the Absence of Inhibitors

It now seemed that the explanation of the failure to show a ouabain effect on steady-state fluxes was related to the appearance of K-dependent K efflux when ouabain was added. It remained necessary to show whether

Table 7. Effect of step change in serosal potassium on potassium efflux ($n=6$)

	J_{1M}	J_{1S}	A_1	k_{M1}	k_{S1}
Control, [K]=2.5 during load and wash	0.0199 ±0.0082	0.5042 ±0.1089	1.778 ±0.494	0.0100 ±0.0025	0.3489 ±0.0582
Experimental, [K]=2.5 during load [K]=0 during wash				0.0078 ±0.0017	0.4702 ±0.0887
$P_{\text{difference}}$				NS	NS

or not such a mechanism also existed in the absence of ouabain. Experiments were therefore performed on control bladders both loaded and washed into a Ringer's solution containing 2.5 mM potassium, followed by an experimental wash into a medium which contained no potassium. The results of these experiments are shown in Table 7. It can be seen that there is no significant effect of this maneuver in the absence of ouabain, although, as expected (Essig & Leaf, 1963; Finn, Handler & Orloff, 1967), the short-circuit current fell sharply.

Effect of Alterations in Mucosal Sodium on Potassium-Dependent Potassium Efflux

Since we have previously shown that removal of sodium from the mucosal medium causes a significant reduction in the K uptake at the serosal border (Finn & Nellans, 1972), it was necessary to examine the effect of this maneuver on the K-dependent K efflux. A series of experiments was thus performed, and these are shown in Table 8. First of all, as shown in experiments 1 and 2, there was no effect of ouabain alone on any of the steady-state flux parameters in the absence of sodium in the mucosal medium, just as was shown in Table 1 for a normal Ringer's solution. Furthermore, as shown in experiments 1 through 4, there is no evidence for a K-dependent K efflux in the presence of ouabain under these conditions. Thus, washing the tracer out into a medium containing either 10 or 0 mM potassium appears to have no effect on the K efflux rate coefficient k_{S1} in the presence of ouabain. Finally, as shown in experiments 5 through 8, there was also no effect of changes in K concentration on the washout of potassium when no inhibitor was present. Thus the ouabain-dependent, K-sensitive K efflux requires the presence of sodium in the mucosal medium.

Table 8. Effect of ouabain on potassium kinetics in the absence of mucosal sodium

Exp.		J_{1M}	J_{1S}	A_1	k_{M1}	k_{S1}
1	$[Na]_M = 0$	0.0704	0.9524	1.615	0.0436	0.5896
	+ Ouabain 10^{-3} M	0.0736	0.6156	1.436	0.0513	0.4287
	+ Ouabain, washout into [K] = 10				0.0503	0.4629
2	$[Na]_M = 0$	0.0138	0.2020	0.3198	0.0430	0.6316
	+ Ouabain, 10^{-3} M	0.0157	0.4200	0.6047	0.0259	0.6965
	+ Ouabain, washout into [K] = 10				0.0294	0.3749
3	$[Na]_M = 0$, ouabain 10^{-3} M	0.1203	0.4511	3.127	0.0385	0.1442
	Same, washout into [K] = 10				0.0278	0.1400
4	$[Na]_M = 0$, ouabain, 10^{-3} M,					
	Washout into [K] = 10				0.0756	0.2037
	Same, washout into [K] = 0				0.0666	0.2154
5	$[Na]_M = 0$	0.0067	0.1343	1.023	0.0065	0.1313
	Same, washout into [K] = 0				0.0074	0.1842
6	$[Na]_M = 0$	0.0372	0.3889	1.846	0.0201	0.2107
	Same, washout into [K] = 0				0.0265	0.2222
7	$[Na]_M = 0$	0.0173	0.2777	1.554	0.0111	0.1787
	Same, washout into [K] = 0				0.0091	0.1634
8	$[Na]_M = 0$	0.0412	0.9170	2.412	0.0171	0.3802
	Same, washout into [K] = 0				0.0200	0.3817

Discussion

Sodium transport across the isolated toad bladder involves at least a two-step process, namely, entry of sodium at the mucosal border and active transport at the serosal border. The latter has been thought to involve Na–K exchange. However, previous evidence for the existence of such exchange in epithelial tissue has been indirect, owing to the difficulties involved in making measurements of the fluxes at the different borders of the cells. By utilizing the present method, however, we have been able to show that there is in fact such an obligatory coupling mechanism under normal conditions (Finn & Nellans, 1972). Although the data suggested that the coupling ratio was close to 1:1, the technique did not allow for the determination of accurate stoichiometry since both Na and K fluxes could not be determined simultaneously.

Since Na–K coupling also occurs in red blood cells, where ouabain has been shown to inhibit both entry of potassium and exit of sodium (Glynn, 1957), and since ouabain has also been shown to have a marked inhibitory

effect on the Na transport system in toad bladder, we expected to find that the addition of this inhibitor would affect the K fluxes. However, as is shown here, no demonstrable effect was seen on K fluxes in the steady state. It should be pointed out, on the other hand, that it has been clearly shown in toad bladder that the addition of ouabain results in a significant depletion of total tissue potassium (Finn, Handler & Orloff, 1966; Herrera, 1968). In view of subsequent data obtained herein, however, it seems clear now that such a change in total tissue potassium occurs in a compartment other than that involved in epithelial Na–K exchange, such as smooth muscle cells, blood cells, fibrous tissue cells, or even in another compartment within the epithelial cells. In this regard, it should be added that the fall in potassium reported in the presence of ouabain in isolated toad bladder epithelial cells (Lipton & Edelman, 1971; Macknight, DiBona, Leaf & Civan, 1971) must be regarded with considerable caution, since the disruption of the cells (with the consequent loss of those properties conferred on the intact epithelium by the presence of oriented series barriers and intercellular junctions) may well result in changes in cell contents which are independent of the transport function in the intact system. On the other hand, the methods used here yield measurements of that moiety of potassium (and, as previously reported, of sodium) which is involved in the phenomenon of active transepithelial Na transport.

It has been shown previously in red blood cells (Glynn, Lew & Lüthi, 1970) that there is a component of K efflux that is affected by the K concentration of the external media, and that is only partly inhibited by ouabain; hence there is still a K-dependent K efflux seen even in the presence of ouabain. In the case of the toad bladder, present data have clearly shown that there is a component of K efflux into the serosal medium which is dependent upon the concentration of potassium in that medium, which appears to saturate at a level of potassium in the medium of approximately 5 mM, and which is not seen unless ouabain is present.

At this point, it is necessary to consider the technique of measurement of this K efflux rate coefficient. In red blood cells, K flux rates are low enough so that both the efflux rate coefficient and the efflux may be measured, even when they are measured in media containing zero potassium. However, in toad bladder the rate coefficient of K efflux is extremely high, so that there will be a change in cell K concentration under these circumstances; thus, it is impossible, by the present technique (Finn & Rockoff, 1971), to calculate the actual efflux. However, as stated above, determination of the efflux rate coefficient does not depend upon the steady state for tissue potassium, but merely on the steady state for tissue tracer at the time of the

beginning of the washout experiment, a condition which is met in all experiments (Finn & Rockoff, 1971; Finn & Nellans, 1972).

In addition, it must be recalled that the rate coefficient which is changing has a value of approximately 0.4 min^{-1}, or a half time of less than 2 min. Thus, what we are observing with a virtually instantaneous change in serosal potassium not only occurs quite rapidly, but also is observed for only a few minutes owing to the rapid washout of tracer. Therefore, the finding of rather large changes in the efflux rate coefficient (in the presence of ouabain) as the K concentration of the media is changed would suggest that this mechanism of K-dependent K efflux may be considerably larger than that determined in these studies.

Additional characteristics of this K efflux system are as follows: In the first place, the mechanism is inhibited by furosemide, a diuretic agent which inhibits Na transport. That furosemide acts by a different mechanism than ouabain is quite clear; not only does it inhibit the ouabain-dependent K-dependent K efflux, but it also inhibits both K influx and K efflux in the steady state, as shown in Table 5A.

Secondly, serosal K efflux does not appear to be related to external potassium in control preparations (that is, in the absence of ouabain) although the present technique is clearly sensitive enough to detect changes in K fluxes and pools when the steady-state K concentration is changed, as shown in Table 2. In addition, the data in Table 2 make it clear that in the steady state in the absence of inhibitors, neither K efflux rate coefficient is dependent on pool size or on medium potassium, although the fluxes and pool do have such a dependence. Thus, when external K concentration is elevated, a new steady state of pool potassium is reached via an increase in the influx. It is of considerable interest to determine whether this increased influx is mediated by an increase in the Na–K coupled system. Although the short-circuit current appears to increase when the serosal K concentration is increased to 10 mM, determination of the pathway of K entry will require measurement of the appropriate unidirectional Na fluxes as a function of serosal potassium. Such studies are currently under way.

Finally, the K-dependent K efflux which we have described clearly requires the presence of sodium in the mucosal medium, as shown in Table 8.

To explain the present data, we might consider several possible model systems. In the first place, as shown in Fig. 2*A*, one might simply consider that there is normally a Na–K exchange system at the serosal border, which, in the presence of ouabain, changes to a K–K exchange system. This might occur if ouabain alters the affinity of the Na efflux system such that potassium may effectively compete for it. If such were the explanation, one would

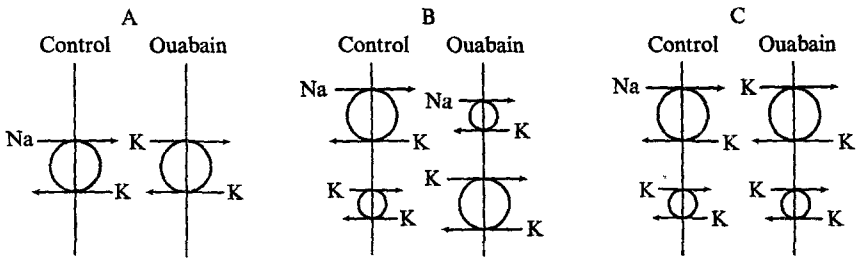

Fig. 2. Models of serosal Na–K transport system. *See* text for discussion

predict that ouabain would not change the size of either the Na or the K transport pool. With regard to potassium, such seems clearly to be the case (Table 1). As far as the Na transport pool is concerned, studies are currently in progress and preliminary data (Finn, *unpublished information*) suggest that ouabain inhibits Na efflux at the serosal border but has little or no effect on the size of the pool. The requirement for mucosal sodium is consistent with this model, since in the absence of sodium, there would be no Na–K exchange even without ouabain; therefore one would not expect to see any K–K exchange when ouabain is added, since the exchange system was shut off, as it were, to begin with. This model, however, does not explain the action of furosemide, which inhibits both ouabain-dependent and ouabain-independent K fluxes.

A slightly different scheme is shown in Fig. 2*B*, in which we suppose that under normal circumstances there is present both a Na–K exchange system and a K–K exchange system. We would then have to assume that the K–K exchange system is too small to be detectable under normal circumstances by the technique employed here. Such is quite possible, since as mentioned, the determination of K efflux into media of different K concentrations may be limited in its sensitivity by the nature of the procedure. According to this model, ouabain inhibits the Na–K exchange system, but in some way stimulates the K–K exchange system so that the latter becomes detectable. This seems to be quite unlikely, since one would not expect ouabain to stimulate a system which is inhibitable by furosemide. Fig. 2*C* represents somewhat of a combination of the two previously mentioned considerations. In this case we again assume that under normal circumstances there exist two separate pathways, a Na–K exchange system and a considerably smaller K–K exchange system. Since ouabain has no effect on the amount of potassium contained in this compartment, we would suppose that, as stated before, ouabain induces an alteration in the affinity of the transport system, such that potassium effectively competes with

sodium for efflux. Furthermore, we would suppose that the requirement of sodium in the external medium is as previously stated; that is, removal of sodium eliminates the Na–K exchange system, so that ouabain would no longer unmask a K–K exchange system[1]. The effects of furosemide may now be readily explained. First of all, this drug inhibits the K–K exchange seen in the presence of ouabain, either by affecting the alteration in affinity of the pump mechanism, or perhaps simply by inhibiting the entire operation of this system. In addition, however, the effect of furosemide in the steady-state experiments (Table 5A), where both influx and efflux are inhibited, suggests that the smaller K–K exchange system also exists under control conditions. Alternatively, of course, furosemide might separately affect both passive K exit and the active K entry of the Na–K exchange system. The likelihood that this drug would specifically inhibit passive K efflux, however, seems remote; were furosemide to affect only the Na–K exchange system, we would not expect to see an alteration in the efflux rate coefficient, but only a change in the influx. Therefore, we interpret the data as indicating the presence of a small K–K exchange system under normal circumstances.

Thus, we have described a new system for the transfer of K in the toad bladder. Under control conditions there appears to be a Na–K exchange pump at the serosal border which has an obligatory coupling ratio of close to one. The K content of the transport compartment is maintained by the operation of this exchange mechanism and by the concomitant and equal loss of this ion across the serosal membrane, perhaps by passive diffusion, as envisioned by Ussing and Zerahn (1951); however, at least a part of the equal and opposite flow of potassium occurs by the furosemide-inhibitable ouabain-independent pathway described above.

When ouabain is present, on the other hand, K influx into the cells from the serosal medium continues (K fluxes at the mucosal side are always extremely small in comparison to those at the serosal side) because it is coupled to potassium—instead of sodium—efflux. We would now assume that the K content of the pool is maintained because at least a part of the potassium that was presumably diffusing outward under control conditions is now diverted to the pathway which is sensitive to serosal potassium.

Finally, it should be recalled that our earlier studies (Finn & Nellans, 1972) revealed that only a small portion of total tissue potassium is involved in Na–K exchange. The present data support the previous model,

1 The implication here is that as sodium activates the Na–K system, so too does it "activate" the K–K system. If such is the case, then both ouabain-dependent K–K exchange and serosal Na efflux might be expected to bear the same relationship to the mucosal Na concentration. Studies to evaluate this possibility are now being carried out.

since changes occurred only in the compartment previously identified as that involved in exchange with sodium.

This work was supported by United States Public Health Service Grant No. AM-15175.

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