Effect of Head Upward Tilting on Unidirectional Na and H_2O Fluxes Across the Canine Ileum[1] (36533)

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Previous experiments in dogs have shown that the ileum responds to head upward tilting by reducing the Na concentration and volume of solution in the lumen compared to a control period (1). It was suggested that the gut was responding to head upward tilting by increasing its ability to absorb Na against a concentration gradient and that H_2O absorption followed Na absorption. It was also suggested that this response was homeostatic in that it tended to restore an apparent decrease in blood volume and/or blood pressure as sensed by volume or baroreceptors in the head and thorax. Stimulation of these receptors initiate many hormonal and cardiovascular responses (2) and no conclusion as to the mechanism of the effects on the ileum were made.

We turned attention to the possibility of direct cardiovascular effects on ileal absorption of salt and H_2O. It is known that small amounts of hydrostatic pressure, applied to the serosal surface of the intestine, can decrease net movement of solute and/or solvent in the mucosal to serosal direction (3, 4). These observations suggested that the converse might also hold, namely, that a relative decrease in serosal hydrostatic pressure caused by head upward tilting and the consequent reduction in arterial blood pressure might allow a relative increase in salt and H_2O transport in the mucosal to serosal direction. Physical forces such as blood pressure and colloid osmotic pressure are known to alter kidney reabsorption of salt and water in a manner consistent with the above mentioned possibility (5).

Materials and Methods. Dogs, fasted 24 hr, were anesthetized with pentobarbital (30 mg/kg). A segment of terminal ileum was flushed with saline (39°) and the openings were sealed with rubber plugs through which a sampling tube and salt bridge (4% agar–1 *M* KCl) protruded into the lumen. The ileal segment was wrapped in gauze and plastic wrap and placed in a saline-filled, Lucite trough maintained at 39°. A mesenteric vein draining the segment was cannulated with polyethylene tubing (as wide as possible to reduce resistance to blood flow) and blood flow was shunted to a femoral vein cannula through a three-way valve. Blood was collected through the valve for 1 min to determine blood flow and to obtain blood samples. Blood flow from the canulated mesenteric vein was multiplied by the number of parallel veins to obtain an estimate of blood flow through the entire gut segment. Heparin (200 units/kg) was injected into the animal at 30 min intervals.

The ileal segment was filled with a known volume of solution (about 20 ml) of either NaCl (0.9%) or NaCl (0.45%)–$MgSO_4 \cdot 7H_2O$ (3.2%). The different solutions were used to increase the range of Na concentration in and Na absorption from the lumen. $MgSO_4$ is a poorly absorbed salt which holds H_2O in the gut lumen. Samples were taken at zero time and at 15 min intervals for 60 min periods. After the control period the animal was tilted head upward at a 45° angle and absorption was measured in a second period. After the end of the second period the animal was returned to the supine position and a third period was carried out. At the end of each period the ileal segment was flushed with saline. At the end of the experiment the gut segment was removed, drained and weighed. Average gut weights were 29.3 and 30.6 g for segments containing NaCl–$MgSO_4$ or NaCl,

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TABLE I. Average Values (± SEM) for Na and H_2O Fluxes Across Dog Ileum Before, During and After Head Upward Tilting.

Solution in lumen	NaCl–$MgSO_4$ ($N = 16$)			NaCl ($N = 8$)		
Period	Control	Head upward tilting	Return to supine	Control	Head upward tilting	Return to supine
[Na] (meq/liter)	80.9 (3.3)	76.4[a] (4.3)	80.9 (3.7)	124.5 (4.0)	117.2[b] (4.5)	116.2[a] (6.5)
Na absorbed (mμEq/min g gut)	— 195 (154)	64[b] (118)	33[a] (101)	325 (113)	510 (116)	765[a] (111)
Na flux out (mμEq/min g gut)	770 (87)	798 (94)	684 (70)	1166 (150)	1061 (186)	1425 (273)
Na flux in (mμEq/min g gut)	966 (116)	734 (188)	651[a] (96)	840 (190)	572[a] (166)	658 (218)
H_2O absorbed (μl/min g gut)	— 3.26 (1.27)	— 0.80[b] (0.87)	— 1.40[a] (0.73)	1.03 (0.87)	2.46 (0.53)	3.60 (0.73)
H_2O flux out (μl/min g gut)	15.9 (1.1)	10.8[c] (0.7)	9.3[c] (1.0)	13.1 (1.3)	10.1[a] (1.5)	12.7 (2.0)
H_2O flux in (μl/min g gut)	19.2 (1.7)	11.5[c] (1.2)	10.6[c] (0.9)	12.2 (1.9)	7.8[c] (1.3)	9.2 (1.8)
3-*O*-CH_3 glucose absorbed (pmoles/min g gut)	1.87 (0.20)	1.40[c] (0.20)	1.33[b] (0.13)	2.06 (0.47)	1.87 (0.40)	2.40 (0.60)
Transmural voltage (mV)	3.5 (1.7)	8.0[b] (1.9)	6.2[b] (2.3)	9.1 (1.9)	8.9 (1.6)	11.9[a] (1.0)
Calcd[d] flux out Na / flux in Na	0.64 (0.03)	0.64 (0.03)	0.66 (0.04)	1.07 (0.03)	1.02 (0.05)	1.06 (0.04)
Av[e] flux out Na / flux in Na	0.80	1.08	1.05	1.38	1.89	2.16
Mean blood pressure (mm Hg)	133 (4)	102[c] (9)	101[c] (5)	128 (5)	105[b] (5)	99[b] (7)
Blood flow (ml/min g gut)	0.63 (0.04)	0.33[c] (0.04)	0.53[a] (0.02)	0.62 (0.01)	0.37[c] (0.02)	0.46[c] (0.01)
Calcd[e] resistance (mm Hg/ml/min g gut)	211	309	190	206	256	215

[a] Based on paired comparisons between values at the same time the differences between control and subsequent periods were significant at the 5%; [b] 1%; and [c] 0.1% level, respectively.

[d] Flux ratio expected for passive diffusion of Na as calculated by the method of Ussing (8).

[e] Based on the average values given in the table.

respectively. Where pertinent, values are expressed per gram of wet gut weight.

The solutions in the lumen also contained the isotopes (New England Nuclear) ^{22}Na (2 μCi/100 ml), $^{3}H_2O$ (30 μCi/100 ml) and 3-*O*-$^{14}CH_3$-glucose (2 μCi/100 ml—no carrier added). Phenol red (8 mg/100 ml) was also present and used as a volume indicator (6). Isotopes were counted in a three-channel liquid scintillation counter with automatic quench correction (Beckman) in a cocktail of toluene (8.5 ml), Beckman Biosolve 3 (1.5

ml) and PPO (0.06 g). Phenol red concentration was determined colorimetrically in basic solution at 560 mμ and then acidified and reread to determine whether other chromogenic compounds were present but none were found. Na concentration was determined by flame photometry.

Arterial blood pressure was monitored with a Bourdon pressure transducer from a catheter threaded up the femoral artery to the level of the superior mesenteric artery and recorded on a polygraph (Narco Biosystems). Blood pressure during tilting was corrected for the hydrostatic pressure difference between the tip of the cannula and the pressure transducer. Transmural voltage was determined between the salt bridge in the lumen and a reference electrode in the trough. Base line potentials were measured before and after each experiment and were less than 0.5 mV and unchanged. Transmural voltage was determined by a high impedance electrometer (Keithly). Transmural voltages are expressed with the serosal surface at zero potential.

Unidirectional and net fluxes were determined by the method of Berger and Steele (7). Na flux ratios, expected on the basis of passive diffusion, were calculated by the method of Ussing (8). Statistical analysis was by *t* test for paired comparisons where the value at a given time in the control period was compared to the value at the same time in subsequent periods. The relationship between variables was determined by correlation and regression analysis.

Results. All average values are given in Table I in the order discussed in this section, first for the effects of head upward tilting and then following return to the supine position. Following head upward tilting, the average [Na] decreased significantly in the luminal solutions, both NaCl–$MgSO_4$ and NaCl, indicating a relative increase in Na over H_2O absorption. There was net secretion of Na into NaCl–$MgSO_4$ in the control period but net reabsorption following head upward tilting and the differences were significant. There was net absorption of Na from NaCl during the control period; following tilting, the amount of Na absorbed was not significantly changed but did tend to increase. The flux of Na out of either the NaCl–$MgSO_4$ or NaCl solution was not significantly changed following tilting. The flux of Na into NaCl decreased significantly following tilting and also tended to decrease into NaCl–$MgSO_4$, but not significantly. As also shown, in the control period the flux of Na out of NaCl is 152% of the Na flux out of NaCl–$MgSO_4$ while the relative [Na] is 154%. Therefore, the Na flux out is proportional to the luminal [Na]. However, the flux of Na into the two solutions is about the same as would be expected if the Na influx represented passive movement from the blood.

There was net secretion of H_2O into NaCl–$MgSO_4$ during the control period but significantly less secretion following head upward tilting. The volume of H_2O absorbed from NaCl was doubled following tilting but the increase was not significant. The flux of H_2O both into and out of both NaCl–$MgSO_4$ and NaCl decreased significantly following tilting. The differences in net H_2O flux between NaCl–$MgSO_4$ and NaCl are primarily due to a greater flux of H_2O into Na Cl–$MgSO_4$ possibly because the poorly absorbed $MgSO_4$ is creating an effective osmotic pressure difference across the mucosa. If should be pointed out that the above effects are, in general, opposite to those seen when no experimental manipulations are carried out, *i.e.*, the effects of time and deterioration of the gut result is slightly increased [Na] and slightly decreased Na and H_2O absorption (9).

The amount of 3-*O*-CH_3-glucose absorption from NaCl–$MgSO_4$ decreased significantly following tilting and the amount absorbed from NaCl was not significantly changed but also tended to decrease. Transmural voltage was significantly increased following tilting when NaCl–$MgSO_4$ was present in the lumen but voltage was unchanged when NaCl solution was present in the lumen. The flux ratios for Na, calculated on the basis that Na was diffusing passively, were not significantly changed following tilting with either NaCl–$MgSO_4$ or NaCl in the lumen. Therefore, the electrochemical driving force exerted on Na was not changed by tilting. The average

observed flux ratio for Na from both solutions tended to increase following tilting and was greater than the calculated flux, suggesting active Na transport. Both mean arterial blood pressure and blood flow through the ileal segment were significantly decreased following tilting while the average resistance to blood flow tended to increase.

After the animal was returned to the supine position, the average [Na] in Na Cl–$MgSO_4$ increased back to control period levels but the [Na] in NaCl remained significantly lower than in the control period. The amount of Na absorbed from both Na Cl–$MgSO_4$ and NaCl remained significantly increased above control values. The flux of Na out of the lumen was not significantly changed from control levels after the animal was returned to the supine position. After the return to the supine position the flux of Na into NaCl–$MgSO_4$ remained at the significantly low level found during tilting but the flux of Na into NaCl was not significantly different from control levels. Net secretion of H_2O into NaCl–$MgSO_4$ remained significantly decreased following return to the supine position while the volume absorbed from NaCl increased threefold above control levels, but due to its large variability and the fewer observations this increase was not significant. The flux of H_2O out of NaCl–$MgSO_4$ remained significantly decreased but the flux of H_2O out of NaCl increased back to control levels. The flux of H_2O into the two solutions paralleled the flux of H_2O out, namely, remained significantly lower into NaCl–$MgSO_4$ solution and rose toward control levels into NaCl solution. The absorption of 3-*O*-CH_3-glucose from NaCl–$MgSO_4$ remained significantly lower than control but absorption from NaCl increased above control values, but not significantly. Transmural voltage increased significantly above control levels following return to the supine position when either NaCl–$MgSO_4$ or NaCl was present in the gut. The calculated passive Na flux ratios were not significantly changed but the average observed flux ratios remained elevated above control levels. Mean arterial blood pressure and blood flow remained at the same significantly lower level as encountered during tilting, even though the dogs had been returned to the supine position and even though the resistance to blood flow decreased back to control levels.

Neither blood flow nor arterial blood pressure correlated significantly with either the net fluxes of Na or H_2O or with the unidirectional fluxes of Na. But both blood flow and arterial blood pressure correlated significantly with both the flux of H_2O into and out of the lumen (Fig. 1).

Discussion. Head upward tilting increases the ability of the gut to absorb Na against a concentration gradient and decreases secretion (increases absorption) of H_2O from NaCl–$MgSO_4$. Similar findings in other experiments led to the conclusion that head upward tilting *per se* was responsible for these effects, possibly through stimulation of baroreceptors or volume receptors (1). However, since the effect on [Na] in NaCl and many of the effects on Na and H_2O fluxes observed during tilting are maintained or increased even after the animal is returned to the supine position, sequelae of head upward tilting are causing the changes in gut absorption. Since blood pressure and ileal blood flow also do not return to control levels following return of the animal to the supine position it is possible that hemodynamic factors are affecting gut absorption either directly or indirectly or that some of the same factors which alter blood pressure and flow also alter gut transport. The lack of blood pressure recovery following return to the supine position is probably due to transudation of fluid from the plasma into the interstitial space caused by increased hydrostatic pressure in the lower extremities during tilting (10) which would be potentiated by the open abdominal cavity and the consequent reduced efficiency of the respiratory pump. Other investigators have also noted that anesthesia reduces the ability of dogs to maintain blood pressure during tilting and to return blood pressure to control levels following their return to the supine position (11). These same factors would also make a precise estimate of normal homeostatic control of gut function difficult.

The unidirectional H_2O fluxes both into

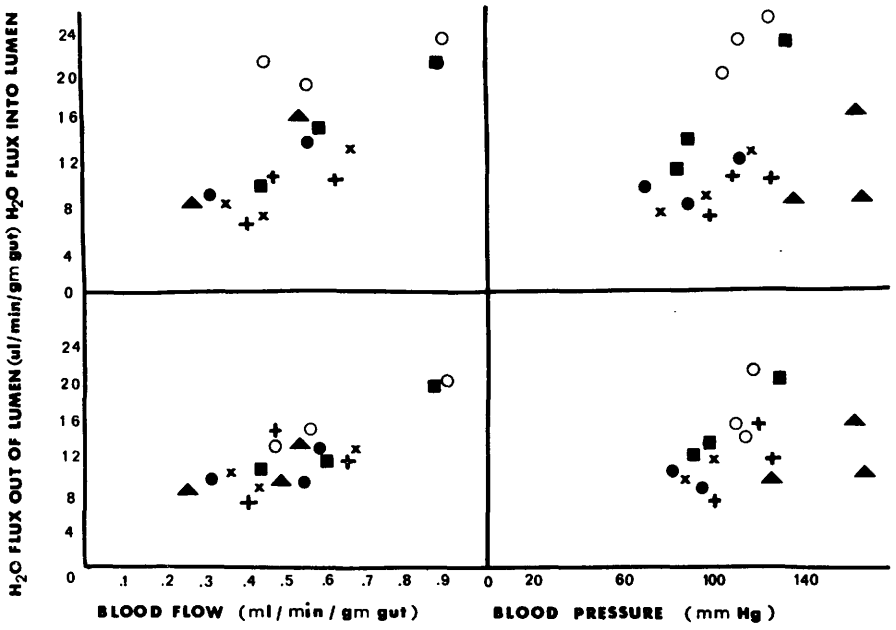

FIG. 1. Relationship between mean blood pressure and blood flow through the gut segment and the flux of H_2O into and out of the lumen. Each symbol represents average of 4 observations in one period and like symbols represent periods in the same dog. Correlation analysis was carried out on the individual observations ($N = 72$). Correlation coefficients and their significance levels were: Blood pressure vs H_2O flux in $r = 0.237$, $p < 5\%$; blood pressure vs H_2O flux out $r = 0.286$, $p < 5\%$; blood flow vs H_2O flux in $r = 0.402$, $p < 1\%$; blood flow vs H_2O flux out $r = 0.402$, $p < 1\%$.

and out of the lumen (but not the net flux) were correlated significantly with both blood flow and blood pressure, suggesting that cardiovascular changes can directly influence H_2O movement across the gut. The solution in the lumen also affects the H_2O fluxes. Following tilting, the increases in Na and H_2O absorption from NaCl–$MgSO_4$ and NaCl were reasonably close to each other but in general only the increased absorption from NaCl–$MgSO_4$ was significant. Possibly, the presence of $MgSO_4$, which artifically lowers the net Na and H_2O fluxes, allows the changes due to the experimental procedures to be more easily detected or elicited. Whatever the manner in which the different solutions affect Na and H_2O fluxes, the cardiovascular changes can still be associated with altered H_2O fluxes. It is possible that the fluxes of H_2O both into and out of the lumen are affected in the same manner by changes in blood flow or blood pressure. For example, intestinal capillaries tend to constrict following decreased blood pressure and their effective surface area is decreased (12) which in turn would tend to reduce both unidirectional H_2O fluxes by decreasing the area available for diffusion between blood and lumen.

Several investigators have shown that increased hydrostatic pressure at the serosal surface of the gut will decrease absorption or increase secretion (3, 4). Decreased blood pressure at the serosal surface could therefore, cause a relative increase in gut absorption. In these experiments, however, the unidirectional H_2O fluxes are better correlated with blood flow than with blood pressure, suggesting that blood flow was the more important parameter. Until estimates of mucosal capillary blood pressure and flow are obtained, no conclusion as to which is the more important hemodynamic factor can be made.

The increased net Na transport which occurred following head upward tilting and/or return to the supine position (depending on

the luminal solution) could not be attributed to specific changes in one of the unidirectional Na fluxes nor could these fluxes be related to hemodynamic factors. Interactions among several mechanisms, some of which tend to decrease Na fluxes and others which tend to increase them, could account for these results. For example, constriction of intestinal capillaries and consequent decreases in their effective surface area (12) would tend to reduce the fluxes of both Na and H_2O both into and out of the lumen. At the same time active Na transport might be stimulated by other mechanisms, perhaps hormonal or related to decreased blood pressure or blood flow. In the above situation the ratio of Na outflux to influx would tend to increase; a 30–50% increase in the average flux ratio for Na was observed in these experiments. The increased Na transport which occurred was not a generalized effect since the absorption of 3-*O*-CH_3-glucose was decreased in periods when Na transport was increased. Furthermore, the increased Na transport could not be attributed to changes in passive electrochemical driving forces since the calculated passive flux ratios for Na were unchanged. That a hormonal effect may be operative is not unlikely since renin is significantly increased within 5 min after head upward tilting (13) and angiotensin (formed by renin) increases fluid transfer across everted rat gut (14).

These results indicate that changes in net and unidirectional Na and H_2O fluxes across the dog ileum occurring after head upward tilting are related to certain prolonged effects of the tilting but the actual mechanisms involved require further studies. In particular, estimates of capillary pressure and effective surface area and possible hormonal influences are needed.

Summary. Head upward tilting and subsequent return to the supine position were both associated with increased net Na and/or H_2O absorption but decreased unidirectional Na and H_2O fluxes across the gut and decreased arterial blood pressure and blood flow through the ileum. The cardiovascular changes correlated significantly with the unidirectional H_2O fluxes both into and out of the lumen but not with net Na or H_2O absorption nor with the unidirectional Na fluxes. It was suggested that vasoconstriction could reduce the capillary surface area available for diffusion and thus reduce the unidirectional fluxes but that additional factors must be affecting Na fluxes.

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