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THE EFFECT OF PROPIONATE AND OTHER ORGANIC ANIONS ON SODIUM TRANSPORT ACROSS TOAD BLADDER

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SUMMARY

1. The electrical effects of isotonic, isohydric solutions of propionate and other organic anions were studied in paired quarter-bladders obtained from the toad, *Bufo marinus*.

2. Serosal propionate solutions produced large, reversible increases in transepithelial potential and short-circuit current, associated with a decrease in electrical resistance. These pH-dependent propionate effects persisted for several hours, and were greatest at concentrations of 66–88 mM. Serosal acetate, butyrate and pyruvate elicited similar responses, but of lesser magnitude.

3. Isotonic concentrations of mucosal propionate slightly reduced the transepithelial potential and short-circuit current.

4. Cationic substitution, $^{24}Na^+$ flux, and O_2-requirement studies demonstrated that the increased short-circuit current induced by serosal propionate was caused by an acceleration of O_2-dependent Na^+ transport from the mucosal to the serosal medium.

5. The electrical effects of serosal propionate and vasopressin were independent.

INTRODUCTION

The urinary bladder of the toad can effect a net transport of Na^+ from the mucosal to the serosal medium in the absence of measured gradients of either electrical potential or Na^+ concentration[1]. This transfer of positive charge is thought to be the basis of the characteristically observed transepithelial potential difference. The electrical potential would be reduced to the extent that anions of the bathing media might also readily traverse the preparation. It might then be anticipated that the transepithelial potential would be inversely dependent upon anion mobility across the preparation. However, toad bladder appears to provide a substantially greater resistance to transepithelial movement of anions than to transepithelial movement of Na^+ (ref. 1). Under these circumstances, the effects of specific interactions of anions with charged groups within the tissue might be of considerably greater significance than the effect of the relative anionic mobilities. By studying the effects of anion substitution on the transepithelial potential, it might then be possible to obtain

information concerning the nature of the predominant charged groups within the preparation[2], as has been successfully carried out with other tissues[3].

Studies of the effects of anion substitution were therefore initiated with anions of very different mobilities. Preliminary experiments with toad bladder revealed that although substitution of propionate for Cl^- in the mucosal medium resulted in a modest fall in transepithelial potential, the same substitution in the serosal medium produced a surprisingly large reversible increase in potential. If the electrical changes were to reflect specific effects on Na^+ transport, serosal propionate might then provide a useful probe in the further study of the underlying mechanism of Na^+ transport, whether the mode of anion action was primarily a facilitation of Na^+ entry into the tissue or primarily a stimulation of Na^+ extrusion from the tissue into the serosal medium.

Some consideration must be given to the possibility that these organic anions participate directly in metabolic processes. Hypotheses concerning the source of the required energy and the mechanism for coupling metabolic energy with transport have been based on the effects of glucose and a variety of organic anion substrates metabolized by the bladder. Most studies of these effects have been conducted at concentrations up to 10 mM in the bathing media[4,5]. When no electrical or metabolic effects are observed at these "substrate-level" concentrations, it is generally assumed that the anions have little or no effect on the ion processes which take place. However, there have been no previous studies in which the organic anion is the major anion present in the medium.

For these reasons, the effects of propionate and some other organic anions, in concentrations up to 110 mM, were studied in paired quarter-bladders, with measurement of the transepithelial potential and short-circuit current.

The results indicate that large serosal concentrations of propionate, and to a lesser extent other organic anions, specifically increase mucosal to serosal movement of Na^+, and that this stimulation is dependent on the presence of O_2. A preliminary report of the studies has been presented elsewhere[6].

MATERIALS AND METHODS

Urinary bladders were excised from doubly-pithed female toads, *Bufo marinus*, obtained from the Dominican Republic (National Reagents, Bridgeport, Conn.). The excised hemibladders were placed in continuously aerated amphibian Ringer's solution (111.4 mM NaCl; 3.5 mM KCl; 2.4 mM $NaHCO_3$; 0.88 mM $CaCl_2$; pH 7.8–8.2; osmolality, 210–230 mosmoles/kg water) at room temperature (23–25°) for varying times prior to use. They were subsequently mounted in a Lucite double-chamber (cross-sectional compartment areas of 1.3 cm^2), so that a single hemibladder provided an experimental and a control quarter-bladder[5]. In all experiments, each compartment was continuously stirred by gassing.

The transepithelial potential difference (V) and the short-circuit current (I) across each quarter-bladder were measured intermittently by methods described previously[6], using Keithley 200 B electrometers and a Weston 622 microammeter, respectively. The tissue resistance (R) was calculated from the ratio V/I, since this relationship is linear over the range studied[7]. Hemibladders were continuously short-circuited during experiments used to study the unidirectional flux of isotopic Na^+.

The solutions in each compartment (volume, 5.0 ml) of the double-chamber were changed by draining the compartment and refilling with the next solution. NaCl–Ringer's solution contained: 104.1 mM NaCl; 3.6 mM KCl; 0.7 mM $CaCl_2$; 0.7 mM Na_2HPO_4; pH 7.8–8.2; osmolality, 220 mosmoles/kg water. Other solutions were made by isotonic, isohydric substitution for either Na^+ or Cl^-, or both, as appropriate to the experimental conditions. (Sodium salts of propionic and pyruvic acids were obtained from Sigma Chemical Co., St. Louis, Mo.; choline salts of propionic acid were made with choline bicarbonate obtained from Hoffman-Taff, Springfield, Mo.)

For the hormonal studies, vasopressin (Pitressin, 20 units per ml; Parke, Davis and Co., Detroit) was added to a final concentration of 100 munits per ml medium in one series of experiments, and (+)-aldosterone (courtesy of Dr. M. M. Pechet) in a methanol carrier was added to a final concentration of 0.1 μM in another series.

The $^{24}NaCl$ used for Na^+ flux measurements was obtained from Cambridge Nuclear Corp., Cambridge, Mass.

RESULTS

Effect of sodium propionate in the serosal medium

The effects of substituting isotonic sodium propionate–Ringer's solution for NaCl–Ringer's solution at the serosal surface of the toad bladder was studied in 10 quarter-bladders. In the initial period of each experiment, NaCl–Ringer's solution was used to rinse and fill both mucosal and both serosal compartments. Subsequently, sodium propionate–Ringer's solution was used to replace the solution in the serosal compartment of the experimental quarter-bladder, while fresh NaCl–Ringer's solution was substituted in the serosal compartment of the control. After development of the peak response to propionate, both serosal media were replaced with fresh NaCl–Ringer's solution. The standard protocol is summarized below.

Quarter-bladder	*Ringer's solution*		
Period:	(1) *Initial*	(2) *Experimental*	(3) *Final*
Experimental			
Mucosal compartment	NaCl	NaCl	NaCl
Serosal compartment	NaCl	Sodium propionate	NaCl
Control			
Mucosal compartment	NaCl	NaCl	NaCl
Serosal compartment	NaCl	NaCl	NaCl

The relative magnitudes of the electrical effects were determined by averaging the initial and final periods to obtain a baseline for each quarter-bladder, and then calculating the percent increases referred to this baseline for the peak propionate effect; the percent changes for the controls were then subtracted from those of the experimental quarter-bladders Within 2 min following the introduction of serosal sodium propionate–Ringer's solution, both transepithelial potential (V) and short-

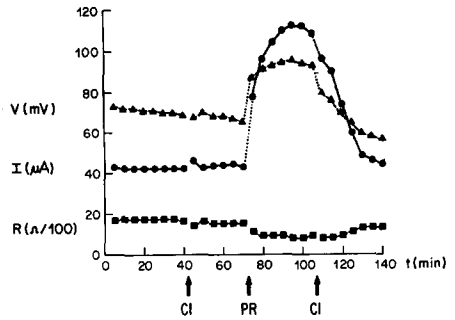

Fig. 1. Electrical response to serosal propionate. V (mV), I (μA), and transepithelial resistance (R, Ω/100) for a typical experimental quarter-bladder are indicated on the ordinate. The time (t, min) is indicated on the abscissa, and the times of changing the serosal solutions are indicated by the arrows. The mucosal compartments contained NaCl–Ringer's solution at all times. (The simultaneous control quarter-bladder showed no changes when the serosal medium was replaced with fresh NaCl–Ringer's solution at each time indicated by the arrows, and has been omitted for clarity.) When the initial NaCl–Ringer's solution was replaced with a fresh solution of the same composition at $t = 42$ (arrow, Cl), the mechanical process produced little change. However, when the serosal solution was next replaced with an isotonic, isohydric sodium propionate–Ringer's solution (arrow, PR), there were large changes in the electrical properties of the quarter-bladder. When the serosal medium was then replaced with fresh NaCl–Ringer's solution (arrow, Cl; $t = 107$) the electrical effects were reversed.

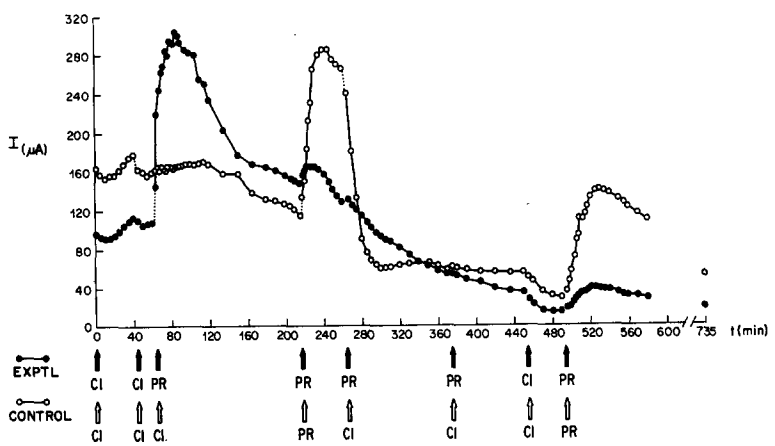

Fig. 2. Duration and reversibility of the electrical response to serosal propionate. I (μA) is indicated on the ordinate for the experimental (solid circles) and control (open circles) quarter-bladders. The time (t, min) is indicated on the abscissa, with the times of serosal solution change indicated by the arrows. The mucosal compartments contained NaCl–Ringer's solution at all times. After an initial exchange with fresh NaCl–Ringer's solution at $t = 42$, the serosal medium of the experimental quarter-bladder was replaced with an isotonic, isohydric sodium propionate–Ringer's solution at $t = 62$ (arrow, PR). This exchange resulted in a large increase in I, which declined slowly after a peak was reached ($t = 85$), and was not increased further by repeated exchanges with fresh sodium propionate–Ringer's solution ($t = 216$, 262, 372). After 6.5 h of exposure to serosal propionate ($t = 452$), the serosal medium was replaced with fresh NaCl–Ringer's solution (arrow, Cl). This exchange resulted in a fall in I, which was reversed by the addition of fresh sodium propionate–Ringer's solution ($t = 495$), and demonstrated persistence of the I response to serosal propionate for more than 6 h. The validity of the paired control quarter-bladder as a parallel control was demonstrated by the large reversible response to serosal sodium propionate–Ringer's solution at $t = 216$ (arrow, PR), and the comparable response which was elicited at the end of the experiment ($t = 495$; arrow, PR).

circuit current (I) increased (Fig. 1); maximum values were obtained in 20 ± 2.6 min (mean ± S.E.). V increased by 84 ± 11%, and I increased by 147 ± 16%; therefore, tissue resistance decreased. When the sodium propionate–Ringer's solution was washed out in the final period, these electrical effects were found to be reversible. In some cases, the electrical response to serosal propionate was found to last for several hours, both at high concentrations (Fig. 2, experimental) and at low concentrations (Fig. 3, control).

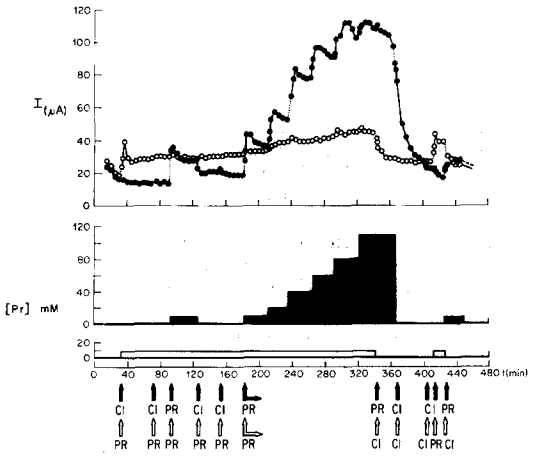

Fig. 3. Effect of serosal propionate concentration on short-circuit current. I (μA) is indicated by the curves on the upper ordinate scale; the serosal propionate concentrations ([Pr], mM) are indicated by the bar graphs on the lower ordinate scale, with the times of serosal solution change indicated by the bar graph steps and the arrows below. Values for the experimental quarter-bladder are indicated by the solid symbols (circles, bar graphs, arrows), and control quarter-bladder values by the open symbols. The time (t, min) is given on the common abscissa. The mucosal compartments contained NaCl–Ringer's solution (Cl) at all times. The initial I was well-matched in the original NaCl–Ringer's solution, with no propionate present. When the propionate concentration in the serosal medium of each quarter-bladder was increased from 0 to 10 mM (control, $t = 31$; experimental, $t = 91$), I increased about equally. Successive replacements of the serosal medium of the control quarter-bladder with similar fresh solutions produced little further change. However, replacement of the serosal medium of the experimental quarter-bladder with solutions of successively increasing propionate concentration (10–110 mM) produced successive increments in I, with the maximum reached at about 80 mM propionate. In each quarter-bladder, replacement of the sodium propionate–Ringer's solution (PR) with fresh NaCl–Ringer's solution resulted in a decrease in I and subsequent replacement with sodium propionate–Ringer's solution again elicited a response, demonstrating that the response had lasted at least 6 h from the first propionate administration.

The effects of a second replacement of serosal NaCl–Ringer's solution with an isotonic sodium propionate–Ringer's solution are not very different from those of the first such replacement (Figs. 2 and 3; Fig. 6, control). The reintroduction of a concentration of 88 mM or more sodium propionate to the serosal medium of 19 quarter-bladders previously treated with similar concentrations resulted in a 136 ± 22% increase in I, which is little different from the 147 ± 16% increase noted above following the first introduction of sodium propionate.

The concentration-dependence of the serosal propionate effect was studied in a series of 13 experiments in which the sodium propionate concentration was varied in both increasing and decreasing sequences, with both NaCl and sodium propionate

controls. A representative experiment is shown in Fig. 3, where successive increments in the concentration of propionate produced successive increases in I; the maximum response was usually reached between 66 and 88 mM. Although some response was usually demonstrated at a concentration of 10 mM, occasional quarter-bladders demonstrated no significant response until a concentration of 20 mM was reached.

The increases in V and I produced by serosal propionate was strikingly dependent on the pH of the serosal medium. In each of three quarter-bladders, the effects of replacing isotonic NaCl–Ringer's solution with isotonic sodium propionate–Ringer's solution were studied at both pH 8.0 and 7.0, in both increasing and decreasing pH sequences. The electrical effects of serosal propionate were markedly and reversibly reduced at the lower pH.

Effect of isotonic mucosal sodium propionate

The effects of mucosal sodium propionate–Ringer's solution were studied according to the protocol of the previous section except that during the experimental period sodium propionate–Ringer's solution was added to the mucosal rather than the serosal compartment; the magnitudes of the effects on V and I were calculated in a similar manner. From 19 quarter-bladder experiments, it was found that isotonic mucosal sodium propionate–Ringer's solution reduced V by 5.0 $\pm$ 2.4% and reduced I by 13 $\pm$ 2.0%. These effects are illustrated in Fig. 4; note that replacement of both serosal and mucosal media with isotonic sodium propionate–Ringer's solution results in the large, reversible increase in I produced with this solution in the serosal compartment alone. These findings imply that simple bridge asymmetry or junction potentials do not play an important role in the serosal propionate response.

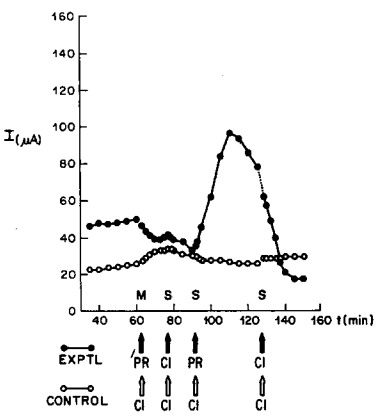

Fig. 4. Comparison of mucosal and serosal propionate responses. I, (μA) is indicated on the ordinate for the experimental (solid symbols) and control (open symbols) quarter-bladders. The time (t, min) is given on the common abscissa, with the times of solution change in the mucosal (M) or serosal (S) compartments indicated by the corresponding arrows below. The initial 35 min of stabilization and rinsing both mucosal and serosal compartments with NaCl–Ringer's solution have been omitted. Replacement of the mucosal NaCl–Ringer's solution (Cl) of the experimental quarter-bladder with an isotonic, isohydric sodium propionate–Ringer's solution (PR) (t = 62) resulted in a fall in both V (not shown) and I. Subsequent replacement of the serosal NaCl–Ringer's solution with a similar sodium propionate–Ringer's solution (t = 91) resulted in large, reversible increases in both V and I, although the mucosal compartment contained the same sodium propionate–Ringer's solution.

Ionic basis of the increased short-circuit current following serosal sodium propionate

Under a variety of circumstances, I has been found equal to the net mucosal-to-serosal Na^+ flux across toad bladder[1]. However, in the present study it was possible that the increases in V and I reflected the development of a propionate diffusion potential across one of the series barriers within the tissue[8,9]. For this reason choline was substituted for Na^+ in three different protocols.

The first protocol compared the effects of serosal propionate successively in choline chloride (low Na^+) and NaCl (high Na^+) solutions, as represented below:

Quarter-bladder	*Ringer's solution*					
Period:	(1) *Initial*	(2) *Exptl.*	(3) *Final*	(4) *Initial*	(5) *Exptl.*	(6) *Final*
Experimental						
Mucosal compartment	Choline chloride	Choline chloride	Choline chloride	NaCl	NaCl	NaCl
Serosal compartment	Choline chloride	Choline-propionate	Choline chloride	NaCl	Sodium propionate	NaCl
Control						
Mucosal compartment	Choline chloride	Choline chloride	Choline chloride	NaCl	NaCl	NaCl
Serosal compartment	Choline chloride	Choline chloride	Choline chloride	NaCl	NaCl	NaCl

The absolute magnitudes of the electrical effects were calculated for each quarter-bladder in each experimental period as the difference between the peak effect in the experimental period and the average baseline determined from the corresponding initial and final periods. The relative magnitudes were then determined as the percent increases referred to the initial NaCl baseline (Period 4); the differences between the experimental and control quarter-bladders were then calculated. For the 4 paired quarter-bladders studied with this protocol (Fig. 5), serosal propionate increased V by 93 ± 27 % in high Na^+ solutions and by 22 ± 9.0 % in low Na^+ solutions. I increased by 153 ± 28 and 8.0 ± 9.0 % in solutions containing high and low Na^+ concentrations, respectively. These results demonstrate that Na^+ is required for the propionate response.

The second protocol was designed to study the Na^+ requirement at the mucosal surface alone. Responses to serosal sodium propionate–Ringer's solution in the presence of mucosal choline chloride were compared to those with mucosal NaCl–Ringer's solutions (see following page).

The relative increments in V and I were calculated as in the preceding protocol. In 3 paired quarter-bladder experiments, reduction of the mucosal Na^+ concentration (choline chloride–Ringer's solution) reduced the response to serosal sodium propionate–Ringer's solution. With low mucosal Na^+ solutions in the first sequence (Periods 1–3) for the experimental quarter-bladder, the increases in V and I were 54–88 and 19–52 % respectively; on the other hand, the simultaneous controls with high mucosal Na^+ solutions demonstrated V and I increases of 133–400 and 177–288 %, respectively. The second experimental sequence (Periods 4–6) demonstrated that the experimental and control quarter-bladders give comparable large responses

Quarter bladder	*Ringer's solution*					
Period:	(1) *Initial*	(2) *Exptl.*	(3) *Final*	(4) *Initial*	(5) *Exptl.*	(6) *Final*
Experimental						
Mucosal compartment	Choline chloride	Choline chloride	Choline chloride	NaCl	NaCl	NaCl
Serosal compartment	NaCl	Sodium propionate	NaCl	NaCl	Sodium propionate	NaCl
Control						
Mucosal compartment	NaCl	NaCl	NaCl	NaCl	NaCl	NaCl
Serosal compartment	NaCl	Sodium propionate	NaCl	NaCl	Sodium propionate	NaCl

with high mucosal Na^+ concentrations. The potential and current increments for the experimental quarter-bladders were 62–114 and 58–100 %, respectively, whereas those for the controls were 46–267 and 36–192 %, respectively. (This protocol is not illustrated in the figure.)

The third protocol was designed to study the Na^+ requirement at the serosal surface alone. This protocol was identical to the second, except for interchanging the mucosal and serosal cations in the first experimental sequence (Periods 1–3) for the 2 experimental quarter-bladders (Fig. 5). The serosal propionate solutions produced

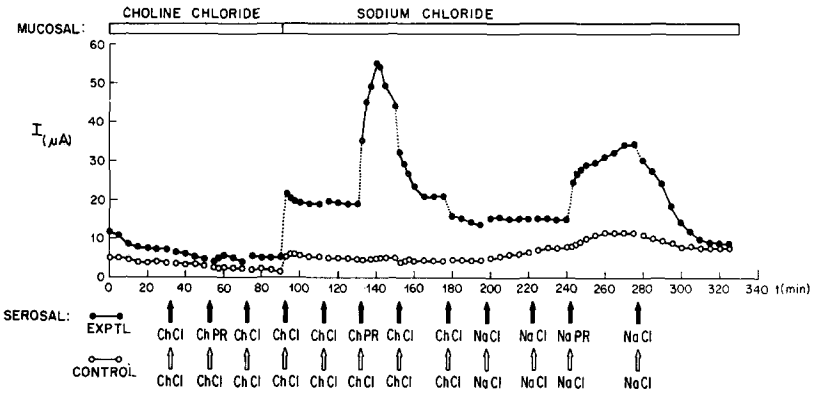

Fig. 5. Effects of Na^+ and choline on the response to serosal propionate. I (μA) is indicated on the ordinate for the experimental (solid symbols) and control (open symbols) quarter-bladders. The time (t, min) is given on the common abscissa, with the times of serosal solution change indicated by the corresponding arrows below. The major component of the mucosal Ringer's solution is indicated by the bar graph at the top, with the vertical line (t = 91) indicating the time of change. Note that when Na^+ replaced choline in the mucosal medium there was an increase in the baseline I, but that there was little further change with serosal Na^+ (t = 197). When the mucosal compartment contained choline chloride–Ringer's solution (ChCl), replacement of the serosal choline chloride–Ringer's solution of the experimental quarter-bladder with isotonic, isohydric choline propionate–Ringer's solution (ChPR) (t = 52) produced little or no change in V (not shown) or I. After replacement of the mucosal choline chloride–Ringer's solution with an isotonic, isohydric NaCl–Ringer's solution (NaCl) (t = 91), a second replacement of serosal choline chloride–Ringer's solution with choline propionate–Ringer's solution (t = 132) did result in a large increase in V and I. The use of serosal isotonic, isohydric sodium propionate–Ringer's solution (t = 242) in a third experimental sequence was not more effective than choline propionate–Ringer's solution under these mucosal conditions.

similar increases in V and I, whether or not Na^+ was present in the serosal medium. With the results obtained in the preceding two protocols, it seems clear that Na^+ is required primarily at the mucosal surface.

In addition to the cation substitution experiments, the role of Na^+ in the response to serosal propionate was investigated in 10 quarter-bladder experiments by simultaneous measurement of the mucosal-to-serosal flux of $^{24}Na^+$ and the magnitude of the currents in continuously short-circuited preparations. After a brief period of stabilization, the isotope was introduced into the mucosal medium with all compartments containing NaCl–Ringer's solution. After a 30-min control period, sodium propionate–Ringer's solution was used to replace the serosal medium. The differences between the $^{24}Na^+$ flux increments for the experimental and control periods were then compared to the corresponding differences in I estimated by planimetry. No significant difference was found between the flux increments (mean: 0.86 μequiv/30 min per quarter-bladder) and the current increments (mean: 0.88 μequiv/30 min per quarter-bladder); the mean paired difference was 0.02 $\pm$ 0.06 μequiv/30 min per quarter-bladder ($P > 0.7$).

Relationship of the serosal propionate effect to metabolism

In order to investigate the relationship of aerobic metabolism to the effect of serosal propionate, 5 paired quarter-bladders were studied sequentially under anaerobic (N_2) and aerobic (O_2) conditions, according to the following protocol:

Quarter bladder	*Period*: (1) *Initial*	(2) *Exptl.*	(3) *Final*	(4) *Initial*	(5) *Exptl.*	(6) *Final*
Experimental						
Mucosal compartment	N_2: NaCl	NaCl	NaCl	O_2: NaCl	NaCl	NaCl
Serosal compartment	N_2: NaCl	Sodium propionate	NaCl	O_2: NaCl	Sodium propionate	NaCl
Control						
Mucosal compartment	O_2: NaCl	NaCl	NaCl	O_2: NaCl	NaCl	NaCl
Serosal compartment	O_2: NaCl	Sodium propionate	NaCl	O_2: NaCl	Sodium propionate	NaCl

All solutions prepared for the anaerobic experimental quarter-bladders and each of the chamber compartments concerned were continuously bubbled with N_2 (Periods 1–3). The control quarter-bladders, and the experimental quarter-bladders under aerobic conditions (Periods 4–6) were aerated continuously in the usual manner. The addition of isotonic sodium propionate–Ringer's solution to the serosal compartment of sufficiently O_2-deficient tissues produced little or no change in V or I while the control quarter-bladders responded in the usual manner. After restoration of O_2 to the previously anaerobic experimental bladders, the experimental and the control bladders exhibited comparable propionate responses (Fig. 6). The relative increments in V and I were calculated with reference to the initial NaCl baseline under aerobic conditions (Period 4). The differences between the experimental and control propionate responses for V and I were only 1.0 $\pm$ 7.1 and 5.0 $\pm$ 13 %, respectively, under aerobic conditions (Period 5); but these differences in V and I were 82 $\pm$ 16

and 152 ± 16 %, respectively, under anaerobic conditions, and are comparable to those observed between sodium propionate-treated quarter-bladders and their NaCl controls (84 ± 11 and 147 ± 16 %, for V and I, respectively).

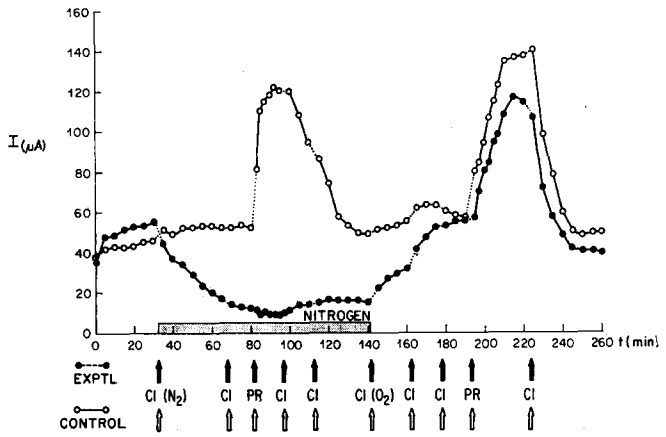

Fig. 6. Effect of anoxia on the electrical response to serosal propionate. The scales and symbols are similar to those in previous figures. The duration of the anoxic (N_2) period is indicated by the shaded bar graph above the abscissa, and by the N_2 and O_2 symbols adjacent to the arrows for the experimental quarter-bladder. The control quarter-bladder underwent the same solution changes indicated by the arrows, but was always aerated. The mucosal compartments always contained NaCl–Ringer's solution. After the baseline I of the experimental quarter-bladder was reduced to a low level by maintenance in a N_2 atmosphere (t = 32), the serosal NaCl–Ringer's solution (Cl) of each quarter-bladder was replaced with an isotonic, isohydric sodium propionate–Ringer's solution (PR) (t = 82). The control quarter-bladder exhibited a typical reversible electrical response, but the anoxic experimental quarter-bladder showed little or no change. When O_2 was returned to the experimental quarter-bladder (t = 142) the baseline I increased to a level comparable to the pre-anoxic level, a level comparable to that of the control quarter-bladder in each case. Subsequent replacement of both serosal NaCl–Ringer's solutions with an isotonic, isohydric sodium propionate–Ringer's solution (t = 192) resulted in similar large and reversible increases in V (not shown) and I. Note that these responses were also comparable to the first response elicited from the control quarter-bladder.

The effects of other organic anions were also examined to determine whether the effect of serosal propionate was shared by other substrates metabolized by the bladder. Protocols similar to that for isotonic serosal sodium propionate–Ringer's solution in the first section were used for 4 experiments with sodium acetate and for 3 experiments with sodium butyrate; calculations of the relative increments in V and I were performed in an analogous manner. Isotonic serosal sodium acetate–Ringer's solution was found to produce increases of 40 ± 7.5 and 59 ± 15 %, respectively, in V and I; isotonic serosal sodium butyrate–Ringer's solution produced increments of 52 ± 16 and 44 ± 31 %, respectively. Representative sodium acetate and sodium butyrate responses are shown in Fig. 7, which also demonstrates that the presence of serosal Cl^- was not required for these responses; the sodium acetate response was obtained with an NaBr–Ringer's rather than NaCl–Ringer's solution baseline (Fig. 7A).

Isotonic serosal sodium pyruvate also increased V and I, but considerably less than serosal propionate in the same tissues (Fig. 8). Protocols similar to those in the first section were used for 5 paired quarter-bladder experiments, except that sodium

pyruvate–Ringer's solution was used in the experimental period for the control quarter-bladders; the relative increments in V and I were determined from the averaged baseline obtained from the initial and final periods for each quarter-bladder.

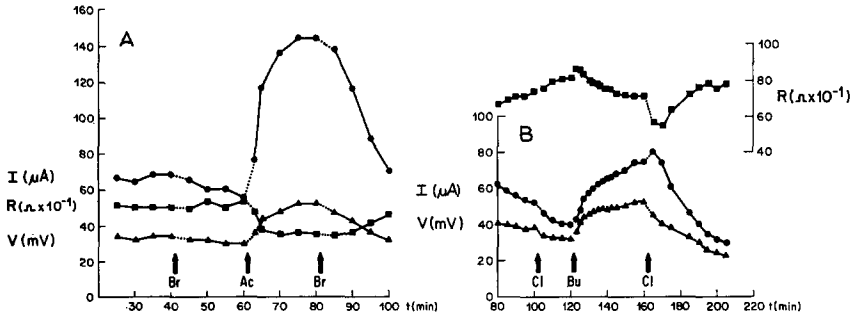

Fig. 7. Electrical responses to serosal acetate and butyrate. Symbols and scales are the same as those in Fig. 1. The mucosal compartments contained NaCl–Ringer's solution. (The control quarter-bladders have been omitted for clarity.) A. Acetate. Although the baseline serosal medium was NaBr–Ringer's solution (Br), reversible increases in V and I were obtained following isotonic, isohydric sodium acetate–Ringer's solution (Ac) which were similar to those observed with a NaCl–Ringer's solution baseline. B. Butyrate. The electrical response to isotonic, isohydric sodium butyrate–Ringer's solution (Bu) was generally of smaller magnitude than those obtained with propionate or acetate.

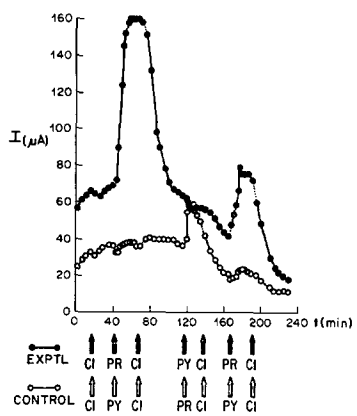

Fig. 8. Comparison of serosal propionate and pyruvate. The scales and symbols are similar to those in other figures. The mucosal compartments contained NaCl–Ringer's solution at all times. The experimental quarter-bladder demonstrated large reversible responses to isotonic, isohydric, serosal sodium propionate–Ringer's solution (PR) both before ($t = 42$) and after ($t = 167$) an isotonic, isohydric sodium pyruvate–Ringer's solution (PY) produced little or no response ($t = 117$). The control quarter-bladder demonstrated simultaneously that sodium propionate–Ringer's solution elicited a reversible response ($t = 117$) both after ($t = 42$) and before ($t = 167$) sodium pyruvate–Ringer's solution produced little or no response.

The respective increases in V and I were 30 ± 6.5 and 24 ± 9.1 % with sodium pyruvate, but were 55 ± 9.1 and 109 ± 18 % with sodium propionate. In 3 experiments with varying concentrations of sodium pyruvate it was found that the successive increments in pyruvate concentration produced small increments in V and I.

These experiments demonstrate that O_2 is required for the response to serosal propionate, and that similar effects may be elicited by other organic anions.

Interaction of hormones with serosal propionate

The effects of vasopressin on the response to serosal propionate, and of serosal propionate on the response to vasopressin, are illustrated in Fig. 9, where it is shown that each effect is independent of the other. In order to evaluate the magnitude of the vasopressin response in the presence of serosal propionate, a series of 10 paired quarter-bladders were studied according to the following protocol:

Quarter bladder	*Period*: (1) *Initial*	(2) *Sodium propionate*	(3) *Vasopressin*
Experimental			
Mucosal compartment	NaCl	NaCl	NaCl
Serosal compartment	NaCl	Sodium propionate	+ Vasopressin
Control			
Mucosal compartment	NaCl	NaCl	NaCl
Serosal compartment	NaCl	NaCl	+ Vasopressin

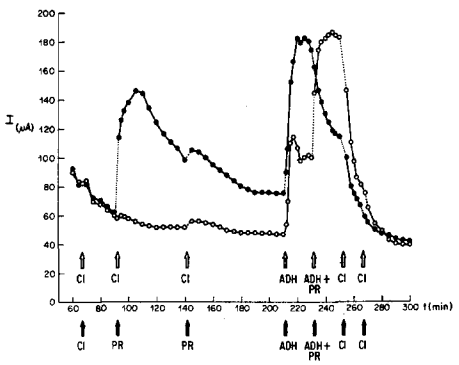

Fig. 9. Interaction of the serosal propionate response with vasopressin. I (μA) for the experimental (solid symbols) and control (open symbols) quarter-bladders are indicated on the ordinate. The serosal solution changes for the experimental quarter-bladder are indicated by the solid arrows below the abscissa, and those for the control are indicated above. The mucosal compartments contained NaCl–Ringer's solution at all times. A large increase in I was elicited by replacement of the serosal NaCl–Ringer's solution (Cl) with an isotonic, isohydric sodium propionate–Ringer's solution (PR) (t = 92). This response was not increased further by a second such replacement (t = 142), and was maintained above the previously well-matched control for more than 2 h. At this time (t = 211) vasopressin (ADH) was added to both serosal compartments, producing more than a 100% increase in I in each case. Subsequently, the serosal medium of the control quarter-bladder (NaCl–Ringer's solution + vasopressin) was replaced by an isotonic, isohydric sodium propionate–Ringer's solution (open arrow, t = 232) containing the same concentration of vasopressin. This exchange resulted in a further increase in I (t = 245), to approximately the same level reached by the experimental quarter-bladder under the same conditions (t = 225). Simultaneously, the serosal medium of the experimental quarter-bladder was replaced with fresh vasopressin-containing, sodium propionate–Ringer's solution (t = 232), which produced no further increase in I. After repeated washout of both serosal compartments with vasopressin-free, NaCl–Ringer's solution (t = 252, 267), I again returned to comparable baselines.

After the development of the peak propionate response (Period 2), vasopressin was added to the serosal compartment of each quarter-bladder (Period 3). The absolute increment in I produced by vasopressin was taken as the difference between the peak

propionate current and the peak vasopressin current. The relative (%) increments were then calculated with reference to the initial NaCl baseline (Period 1). The relative increments in I produced by vasopressin were 88 ± 10 and 97 ± 13 %, respectively, for the experimental (sodium propionate) and control (NaCl) quarter-bladders. Thus the effects of vasopressin and propionate at the peak of the propionate response are approximately additive.

In order to investigate the possible dependence of the serosal propionate effect on aldosterone, 3 paired quarter-bladders were obtained from toads which had been maintained on 0.6 % NaCl solution for at least 48 h. (+)-Aldosterone was added to the serosal NaCl–Ringer's solution of each experimental quarter-bladder; after the development of the peak aldosterone response, isotonic sodium propionate–Ringer's solution was added to all serosal compartments. Both experimental and control quarter-bladders demonstrated typical propionate responses, indicating that aldosterone is not required for the propionate response.

DISCUSSION

The results demonstrate that the addition of propionate to the serosal medium produces large, reversible increases in both V and I, associated with a fall in the electrical resistance across the toad bladder. These concentration-dependent effects may last for several hours, and are shared to a lesser extent by some other organic anions. Although serosal propionate can stimulate a mean increase in I of approximately 150 %, the maximum response may not be reached until a concentration of 66–88 mM. Therefore, the use of low concentrations of serosal propionate might not elicit a response; the use of low pH, or the simultaneous introduction of mucosal propionate, would minimize the observed response at any concentration.

The cation substitution, $^{24}Na^+$ flux, and anaerobic experiments demonstrate that the increased I observed after the administration of serosal propionate is due to an acceleration of O_2-dependent Na^+ transport from the mucosal to the serosal medium. However, whether propionate primarily stimulates aerobic metabolism, with a secondary increase in Na^+ transport, or whether propionate directly decreases the resistance to Na^+ movement across any of the series barriers in the tissue, cannot be established from these data. It is most unlikely that the propionate effect results from a non-specific effect such as cell swelling, since the effect can be abolished by anoxia, low pH, or removal of mucosal Na^+ alone.

Mucosal propionate produces a decrease in V and I by an unknown mechanism. However, preliminary ranking of the effects of a large number of anions substituted in the mucosal medium suggest the characteristic sequence of a lyotropic series[2]. If these findings are comparable to those observed in other tissues, interaction of the propionate directly with the macromolecules of the mucosal surface would be one possible interpretation[3,12].

Vasopressin and aldosterone both produce increases in net Na^+ transport which are additive in fresh tissues[13], suggesting that the hormones act on parallel pathways for transepithelial Na^+ movement.

The hormonal studies demonstrate that the effects of propionate and vasopressin on I are independent and that aldosterone is not required to demonstrate the propionate effect at these high propionate concentrations (*cf.* ref. 14).

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REFERENCES

1 A. LEAF, *Ergeb. Physiol. Biol. Chem. Exptl. Pharmakol.*, 56 (1965) 216.
2 I. SINGER, *Physiol.*, 12 (1969) 356.
3 I. SINGER AND I. TASAKI, in D. CHAPMAN, *Biological Membranes*, Academic Press, London, 1968, p. 347.
4 G. W. G. SHARP AND A. LEAF, *Recent Progr. Hormone Res.*, 22 (1966) 431.
5 R. H. MAFFLY AND I. S. EDELMAN, *J. Gen. Physiol.*, 46 (1963) 733.
6 I. SINGER AND M. M. CIVAN, *Clin. Res.*, 17 (1969) 447.
7 G. W. G. SHARP AND A. LEAF, *Nature*, 202 (1964) 1185.
8 I. SINGER, M. M. CIVAN, R. F. BADDOUR AND A. LEAF, *Am. J. Physiol.*, 217 (1969) 938.
9 A. FINKELSTEIN, *J. Gen. Physiol.*, 47 (1964) 545.
10 H. S. FRAZIER, *J. Gen. Physiol.*, 45 (1962) 515.
11 M. M. CIVAN AND H. S. FRAZIER, *J. Gen. Physiol.*, 51 (1968) 589.
12 I. TASAKI, I. SINGER AND T. TAKENAKA, *J. Gen. Physiol.*, 48 (1965) 1095.
13 M. Z. FALCHUK AND G. W. G. SHARP, *Biochim. Biophys. Acta*, 153 (1968) 706.
14 G. W. G. SHARP AND A. LEAF, *Physiol. Rev.*, 46 (1966) 593.