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EFFECTS OF EVERSION ON TRANSMURAL ELECTRICAL PROPERTIES OF RAT JEJUNUM

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SUMMARY

Transmural potential difference, short circuit current, and electrical resistance were all appreciably greater when measured from noneverted rat jejunum *in vitro* than from everted jejunum. Everting the small intestine apparently decreases active ion transport but increases passive permeability. During the first few minutes of incubation at 37° (following preparation at 0°), potential difference rapidly declined with both everted and noneverted orientations. This decline was caused entirely by a drop in net ion fluxes, since during this time electrical resistance either remained constant or slightly increased. Adequate interpretation of these results must await further study. In the meantime it is worth knowing that important transport properties of intestine are drastically influenced by the common experimental expedient of eversion.

INTRODUCTION

We previously showed that transmural electrical potential difference (PD) across rat jejunum is influenced by everting the intestine[1]. Noneverted segments maintained a considerably higher PD *in vitro* than did everted segments. The PD recorded from noneverted jejunum *in vitro* was similar in magnitude to that recorded *in vivo*. Even with everted segments, transmural PD was fairly high (about 5 mV) immediately after warming to 37° (following several minutes at 0° during preparation), but this initially high PD was not maintained; during the subsequent 4 or 5 min, the PD declined to a fairly stable value of about 1.4 mV. Noneverted segments did not fall as rapidly or extensively as everted segments.

We have further investigated this difference between everted and noneverted jejunal segments by measuring short circuit current (I_{sc}) and transmural resistance (R_m) in addition to PD, hoping to determine if the effect of eversion on PD is caused by a change in ion transport, or in tissue resistance, or in both.

MATERIALS AND METHODS

We used adult male albino rats from either Cheek–Jones Co., Houston, Texas, or Holtzman Rat Co., Madison, Wisc. Each rat was fasted 20–24 h and then anes-

Abbreviation: PD, electrical potential difference.

thetized with ether; its small intestine was rinsed with ice-cold Krebs–Ringer bicarbonate solution and removed by cutting at the radix. After the mesentery was cut away, a 6-cm segment of mid-jejunum was tied onto cannulas A and B of the apparatus shown in Fig. 1. In some experiments the segment was everted with a stainless-steel rod before mounting in the apparatus. The inside Ag–AgCl electrode (C) was inserted through upper cannula A and through the intestinal segment until its beaded tip was just within lower cannula B. In this position rubber stopper D fitted tightly into the upper cannula. Cannulation was done in a cold room at 2°–5°. Except for a few seconds while being tied to the lower cannula, the segment was completely immersed in oxygenated ice-cold Krebs–Ringer bicarbonate solution. The whole assembly, secured by lucite block E, was then transferred to 500 ml of oxygenated ice-cold Krebs–Ringer bicarbonate solution in a beaker which was packed in ice.

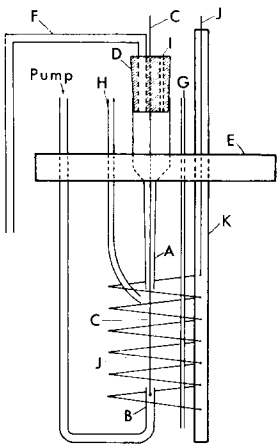

Fig. 1. Diagram of apparatus drawn to scale. A, Upper glass cannula; B, Lower glass cannula; C, Inner Ag–AgCl electrode; D, Rubber stopper; E, Lucite block; F, Outflow tube; G, Gas inlet tube; H, Glass tube for inserting outer PD electrode; I, Hole in rubber stopper for inserting inner PD electrode; J, Outer silver–silver chloride electrode; K, Lucite rod. The distance between the tips of the upper and lower cannulae was 4.5 cm. The Ag–AgCl electrodes were made from 0.50 mm diameter silver wire. The inner electrode was straight with a bead of epoxy on its lower end and was insulated where it passed through the upper cannula (A) and stopper (D); it was sealed in the stopper with latex. The outer Ag–AgCl electrode formed a helix about 4.0 cm in diameter and 1.0 cm between coils; it was insulated over that portion adjacent to lucite rod (K). These electrodes were freshly chlorided before each use.

The lumen of the intestinal segment was perfused with Krebs–Ringer bicarbonate solution from a roller pump (Holter, model RL 155) via the lower cannula. This solution escaped via the upper cannula and outflow tube F; the outlet of the latter was placed outside the 500-ml beaker at a level just above the solution in the beaker, thereby maintaining a very small positive pressure (< 4 cm water) in the lumen. The pumping rate was about 1.5 ml/min. At this flow rate the temperature of solution entering the intestine equalled that in the 500-ml beaker. The perfusate was gassed with 5% CO_2 in O_2 before it entered the pump. The outside solution in the beaker was vigorously gassed with 5% CO_2 in O_2 through tube G.

Electrical measurements were made with techniques similar to those used in previous investigations of everted small intestine[2,3]. For measuring PD, polyethylene

tubes (PE 190) filled with 3 M KCl in 3% agar were inserted into electrode ports H and I. The tips of these electrodes were positioned opposite each other just below the end of the upper cannula. The other ends of these electrodes made contact with calomel electrodes which were connected to a Grass model 5P1 d.c. preamplifier having an input impedance of about $1 \cdot 10^6\ \Omega$. PD was recorded on one channel of a Grass model 5 polygraph. The PD caused by electrode asymmetry was balanced out before each experiment with both electrodes in the same Krebs–Ringer bicarbonate solution. The system was calibrated through the source from the preamplifier.

For measuring I_{sc} Ag–AgCl electrodes C and J were connected to a voltage divider in series with a $10\ \Omega$ precision resistor. Output of the voltage divider was adjusted to give zero PD, while the voltage drop across the $10\ \Omega$ resistor was recorded on a second channel of the polygraph through a second 5P1 preamplifier. A modified Heath recorder (model EUW-20) was used as a servo mechanism which monitored the output of the PD preamplifier and adjusted the voltage divider to permit automatic clamping of the PD at zero (or other desired values). A switching mechanism provided ready alternation between open circuit and short circuit recording.

The incubation medium between the two PD electrodes contributed an appreciable part of the total resistance between these electrodes; therefore, in order to determine the actual I_{sc} and membrane resistance, a correction for "solution resistance" was made. Following each experiment, the intestinal segment was removed, and with the electrodes still in position, current was passed through the Krebs–Ringer bicarbonate solution and the potential drop measured. Transmural electrical resistance (R_m) was estimated as follows:

$$R_m = PD/I_o - PD_s/I_s$$

where PD = open circuit potential, I_o = measured current required to reduce PD to zero, PD_s = potential drop across incubation medium after removing intestine, I_s = current passed through incubation medium after removing intestine.

Short circuit current (I_{sc}) was then calculated by dividing PD by R_m.

After flow was established and a steady PD at 0° was recorded, the entire assembly was quickly transferred to a second 500-ml beaker containing 500 ml of oxygenated Krebs–Ringer bicarbonate solution at 37°. This beaker was immersed in a water bath.

RESULTS

The first few minutes of a typical recording are shown in Fig. 2. Upon transfer from 0° to 37°, both PD and I_{sc} rapidly rose as the temperature rose. Maxima were reached within a minute. These maxima were considerably higher in noneverted than in everted intestine.

All results obtained from 1 to 60 min after warming are compiled in Figs. 3, 4 and 5. PD (Fig. 3) was much higher in noneverted than in everted intestine throughout the entire hour. PD declined sharply from the first to the fourth minute after warming in both everted and noneverted segments, but it then recovered slightly in noneverted intestine to reach a second peak at about 15 min and then fell steadily during the subsequent 45 min. Everted jejunum showed no second peak but merely declined slowly and steadily from about 5 min to 60 min. These PD curves are similar to

those obtained earlier using another technique[1] except that the second peak with noneverted intestine occurred somewhat later in the present experiments and the maximum PD with everted intestine was not as great.

I_{sc} (Fig. 4) was also greater in noneverted than in everted jejunum throughout the entire hour. No second peak was observed in I_{sc} from noneverted intestine. There was no significant change in I_{sc} after about 20 min in either noneverted or everted intestine.

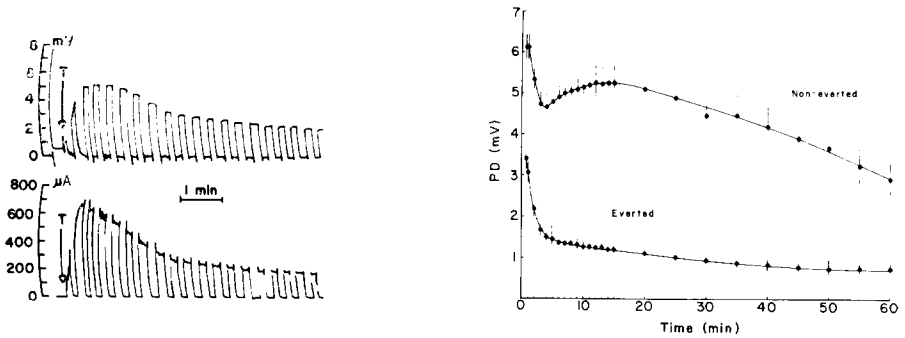

Fig. 2. First few minutes of a typical recording with everted jejunum. Upper tracing is PD, lower tracing is I_{sc}. T marks the time at which the intestinal segment was transferred from 0° to 37°. Transfer took no longer than 2 sec. Recording was alternated every 10 sec between open circuit and short circuit. After the first few minutes of most experiments switching was performed about once each minute. The curved lines used for the ordinate scales indicate the curvature of the lines on the chart paper, which have not been reproduced here. The serosal solution was always positive with respect to the mucosal solution with either everted or noneverted segments.

Fig. 3. PD as a function of time after warming from 0° to 37° for everted and noneverted jejunal segments. At all times to and including 30 min, each point represents the mean from 17 noneverted or 7 everted segments; after 30 min, each point represents the mean from 9 noneverted or 5 everted segments. Each vertical line is the S.E. of the mean. PD values were ascertained from the original records at 1-min intervals until 15 min, and at 5-min intervals thereafter. Whenever the intestine was short circuited at the desired time, PD values were estimated by interpolating between adjacent open circuited periods.

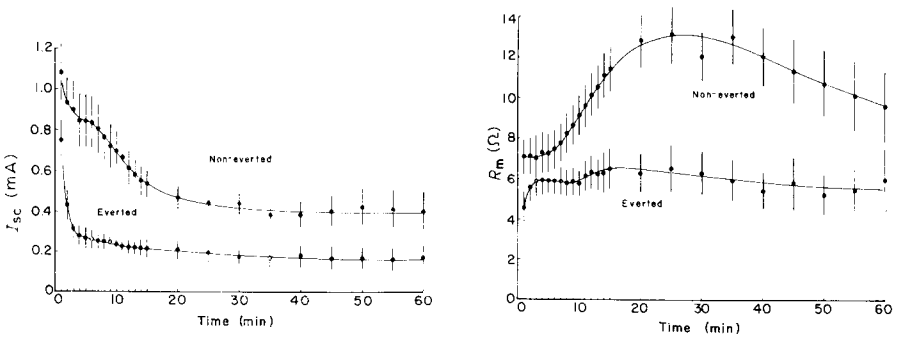

Fig. 4. Short circuit current (I_{sc}) as a function of time after warming. Means ± S.E. are shown. Numbers of animals are the same as in Fig. 3. Whenever the intestine was on open circuit at the time a value for current was desired, we interpolated between adjacent short circuited periods. The values shown have been corrected for solution resistance as described in text.

Fig. 5. Transmural resistance (R_m) as a function of time after warming. Means ± S.E. are shown. Numbers of animals are the same as in Fig. 3. The values shown have been corrected for solution resistance as described in text.

R_m (Fig. 5) was higher in noneverted intestine than in everted intestine throughout the entire hour; however, at 2–7 min and at 60 min the differences had greater than a 5% chance of being caused by random variation according to "t" test. With everted intestine R_m showed a small increase during the first 2 or 3 min but then remained essentially constant for the rest of the experiment. However, with noneverted intestine R_m began to increase at about 6 min. This increase continued until a peak was reached at about 25 min; thereafter, a steady decrease was observed.

As indicated by the standard errors, a considerable amount of animal-to-animal variation was encountered, especially in the R_m measurements. However, the above conclusions were all based on differences which were statistically highly significant.

DISCUSSION

The rapid decrease in PD during the first few minutes after warming rat jejunum to 37° could be caused by a decrease in I_{sc}, or in R_m, or in both. The present experiments demonstrate this early drop in PD to result entirely from a drop in I_{sc}. During this time R_m remained constant with noneverted segments and actually increased slightly with everted segments.

During the entire hour PD, I_{sc}, and R_m were all higher when the jejunum was not everted than when it was everted. Since PD $= I_{sc} \cdot R_m$, the difference in PD was caused partly by a difference in I_{sc} and partly by a difference in R_m. CHALFIN, COOPERSTEIN AND HOGBEN[4] and TIDBALL *et al.*[5] have previously found small intestine to be more permeable when everted than when not everted. Therefore, the decreased electrical resistance of everted intestine was expected. The decreased short circuit current of everted intestine is a new finding and implies an effect on ion transport. Transport of Na^+ across everted rat jejunum in the absence of sugar accounts for essentially the entire short circuit current[3]. In noneverted jejunum either transmural Na^+ transport is increased or transport of some other ion becomes appreciable; a decision on this point must await isotope flux studies.

After roughly 15 or 20 min there was little, if any, further change in I_{sc} for the remainder of the hour with either everted or noneverted segments. The slow decline in PD observed during this time with noneverted segments was caused entirely by a decrease in R_m. This result may be of some practical application. Those investigators who like to ice-down the intestine during preparation before incubation at 37° should expect a few minutes of instability during recovery from the cold. The period from 20 min to 60 min with noneverted intestine or from as early as 5 min to 60 min with everted intestine should be ideal for studying various influences on ion transport and relating ion transport to short circuit current.

With everted segments transmural resistance remained nearly constant from 3 min to 60 min. During this period changes in PD were proportional to changes in I_{sc}. In such a circumstance ΔPD values may be sufficient to characterize the effect of some agent on ion transport if this agent itself has no influence on R_m. LYON AND CRANE[6] used this approach in studying the effect of sugars on ion transport. With noneverted segments transmural resistance was not constant. Changes in PD did not parallel changes in I_{sc}. Even though I_{sc} became reasonably stable in noneverted segments after about 20 min, PD was not stable, and it would not be possible to follow changes in ion transport just by measuring changes in PD. The obvious

instability of the noneverted preparation detracts from its usefulness. However, since it does generate more current than the everted intestine and produces a PD of similar magnitude to that recorded *in vivo*[1], it may be a desirable preparation for some purposes.

NUTBOURNE[7] has shown that very small hydrostatic pressure differences between inside and outside of frog skin influence short circuit current; an increased pressure on the outside increased short circuit current, while increased pressure on the inside had the opposite effect. It might be argued that the difference in short circuit current demonstrated here between everted and noneverted intestine is a similar phenomenon. However, we doubt this interpretation in the case of rat intestine because, when we changed the luminal pressure over a range of about 10 cm of water by elevating or lowering the outflow tube, only very small effects were observed on I_{sc} and PD. The latter were presumably streaming potentials, but they often lasted only a few seconds and were never over a few tenths of a millivolt. NUTBOURNE[7] found no effect on short circuit current by merely bulging frog skin mechanically without a hydrostatic pressure difference. But in the case of intestine, it seems likely that the direction of curvature of the mucosa may be the important factor. Experiments similar to those of NUTBOURNE would be necessary to prove this point.

Explanations for the greater short circuit current and electrical resistance of noneverted intestine are necessarily highly speculative and will not be indulged in extensively here. But it is possible that the brush border of noneverted intestine is more permeable to Na^+ than is that of everted intestine, making it easier for Na^+ to gain access to Na^+ pumps at the lateral and basal plasma membranes. The greater R_m of noneverted intestine could be accounted for if the permeability to ions other than Na^+ (*e.g.*, Cl^-) were decreased. Isotope flux studies would be necessary to evaluate these ideas.

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