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Effects of Bethanechol on Intestinal Ion Transport in the Rat (39599)

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Do the nerves that supply the intestine affect mucosal transport as well as motility? In the studies of Wright *et al.* (1), cutting the splanchnic nerves to the small intestine of the cat caused secretion that was enhanced by the cholinesterase inhibitor, physostigmine, and that was reduced by atropine. Secretion has been elicited in the rabbit ileum *in vitro* with pilocarpine (2), and in the dog jejunum with bethanechol (3). Recently, I studied the effect of pilocarpine on the transport of water and electrolytes by the rat small intestine (4). Pilocarpine is a naturally occurring alkaloid that is unlike acetylcholine in structure but which mimics its muscarinic activity. In the present study, I determined the effect of bethanechol, a compound that is similar in structure to acetylcholine, but which is inactivated at a much slower rate. There are some interesting and unexpected differences in the activities of the two compounds.

Methods. Male Carworth rats weighing 200–300 g were fasted overnight and anesthetized by injecting pentobarbital (35 mg/kg) into the peritoneal cavity. A 20-cm segment of proximal jejunum was cannulated at each end, washed with 20 ml of saline, and flushed with air. A 20-cm segment of distal ileum was similarly treated. A tracheostomy tube was inserted. Solution was perfused once through the segments with a syringe infusion pump at the rate of 0.41 ml/min during two 30-min periods, but fluid was collected for study only during the last 20 min of each period. The input syringe, intestine, and collection syringe attached to the distal cannula formed a closed system which minimized leakage of CO_2. Before each perfusion period, the intestinal lumen was washed with the perfusion solution and was flushed with a mixture of O_2 and 5–6% CO_2 (gas). At the end of each period, collection syringes were removed and capped, and any fluid remaining in the segments was flushed with gas and discarded. After the second period, the segments were removed, stripped of mesentery, and weighed.

The perfusion fluid had the following composition (m*M*): Na = 145, K = 5, HCO_3 = 25, Cl = 125, mannitol = 25. All solutions were gassed and had an initial pH of 7.42–7.43 and PCO_2 of 40–46 Torr. [1,2-^{14}C]Polyethylene glycol ([^{14}C]PEG) was used as a nonabsorbable marker to permit calculation of net water movement.

The pH and PCO_2 of the luminal fluid were determined soon after collection with a capillary pH electrode and PCO_2 electrode designed for small samples. Total CO_2 was measured with Natelson microgasometer. Sodium and potassium concentrations were measured with a flame photometer and chloride was determined with a coulometric chloridometer. The concentration of [^{14}C]-PEG (counts per minute per milliliter) was measured with a scintillation counter.

"Initial" chemical determinations were measured in samples of fluid obtained from the input syringes and samples of fluid for "final" determinations were obtained from the collection syringes after each collection period. Fluid remaining in the segments was discarded.

Net fluxes of ions and water were calculated as follows:

$$J_{net}^{ion} = V\,(ion_f \frac{PEG_i}{PEG_f} - ion_i)gWW^{-1}$$

$$J_{net}^{H_2O} = V\left(\frac{PEG_i}{PEG_f} - 1\right)gWW^{-1}$$

Ion_i and ion_f are the concentrations of the ion (micromoles per milliliter) measured in the initial and final samples; PEG_i and PEG_f are the specific activities of [^{14}C]PEG (counts per minute per milliliter) measured in the initial and final samples. V is the volume (milliliters) of perfusion pumped

into the segment in 20 min; gWW is the wet weight of the intestinal segment in grams. Thus, J_{net}^{ion} is expressed as micromoles × gWW^{-1} × $(20\ min)^{-1}$, and $J_{net}^{H_2O}$ is expressed as milliliters × gWW^{-1} × $(20\ min)^{-1}$. The signs preceding the net flux measurements indicate movement into (+) or out of (−) the lumen.

To measure transmural electrical potential difference (PD), bridges of saturated KCl in agar were used. One bridge contacted the fluid perfusing the intestinal lumen and the other was placed in the peritoneal cavity. Electrical contact with a voltmeter was made through calomel half cells. The voltage (mV) was recorded every 30 sec.

To determine the effect on PD, bethanechol was infused for 10-min periods at increasing rates of 0.24, 0.76, and 2.4 μmoles × kg^{-1} × min^{-1}. After each 10-min period of bethanechol infusion, isotonic saline was infused for 10 min to permit the PD to return to baseline values.

To assess the effect of parenteral bethanechol on ion and water movement, isotonic saline was infused intravenously during the first 30-min perfusion period, and bethanechol was infused during the second at the rate of 2.4 μmoles × kg^{-1} × min^{-1}.

To determine whether bethanechol affected ion and water movement when it was in contact with the external surface of the lumenal cell membrane, bethanechol was added to the intestinal perfusion fluid in the second period in a concentration of 0.5 m*M*. Intravenous bethanechol infusion at 2.4 μmoles × kg^{-1} × min^{-1} at the end of 30 min should produce an extracellular fluid concentration of about 0.5 m*M* assuming that none entered the cells, that there was no degradation, and that extracellular fluid volume was 15% of body weight. Hence, in all likelihood, the concentration of bethanechol in the intestinal perfusion fluid was higher than that attained in the extracellular fluid by intravenous infusion.

The statistical significance of differences between means was determined with a Student's *t* test for paired samples.

Results. Dose response of PD. The negative lumenal PD of the jejunum increased as the infusion rate of bethanechol was increased (Table I). The lowest PD of the control period immediately preceding the infusion was used as the baseline value to calculate the magnitude of the change. At the infusion rate used in the studies of electrolyte and water transport, 2.4 μg × kg^{-1} × min^{-1}, the mean lumenal PD became more negative by 3.3 mV. The PD attained its maximum 6–8 min after the start of the infusion and remained the same throughout the remainder of the period. Because, in the transport studies, the study periods began 10 min after the commencement of the infusion of bethanechol, it is likely that the changes induced by bethanechol were in a steady-state during the period of the study.

At the highest dose, all rats salivated copiously, tears appeared, and most of them urinated or defecated. At the intermediate dose, there was some salivation but at the lowest dose, none of these changes were observed.

Effect of intravenous bethanechol. In the jejunum, bethanechol reduced absorption of Na from 44.0 to 13.8 μmoles × gWW^{-1} × 20 min^{-1} and bicarbonate absorption diminished from 47.7 to 20.9 μmoles × gWW^{-1} × 20 min^{-1} (Table II). Bethanechol increased the final perfusate pH from 7.06 to 7.20 and reduced the final PCO_2 from 70 to 60 Torr.

In the ileum, bethanechol increased the final perfusate pH from 7.40 to 7.43. The large standard deviations in the net movements of Na and Cl in the control studies were caused by one rat which secreted Na and Cl at high rates. If the data from that rat are excluded from the statistical analysis (and $N = 7$), the SD's for Na and Cl net

TABLE I. EFFECT OF BETHANECHOL ON JEJUNAL TRANSMURAL ELECTRICAL PD.[a]

	Bethanechol (μmoles/kg × min^{-1})		
	0.24	0.76	2.4
Δ PD (mV)	−0.3±0.27	−2.1±1.08 ***	−3.3±1.82 **

[a] The mean (± SD) change in jejunal electrical potential difference (PD) caused by intravenous infusion of bethanechol at three different rates. The sign indicates lumenal electrical negativity. The change in magnitude of the PD is related to the PD of the control period immediately preceding the bethanechol infusion; $n = 5$. ** $P < 0.005$; *** $P < 0.001$.

TABLE II. EFFECTS OF BETHANECHOL ON INTESTINAL TRANSPORT.[a]

	Jejunum		Ileum	
	Control	Bethanechol	Control	Bethanechol
Na				
$\bar{X}$	−44.0	−13.8*	−0.1	−6.2
SD	34.3	34.5	61.6	14.8
K				
$\bar{X}$	−0.93	−0.28	+0.96	+0.69
SD	1.37	2.37	1.93	0.73
Cl				
$\bar{X}$	+7.7	+21.0	−24.7	−30.1
SD	20.2	38.9	48.7	17.7
HCO_3				
$\bar{X}$	−47.7	−20.9**	+19.6	+30.7
SD	16.4	21.7	32.2	12.2
H_2O				
$\bar{X}$	−0.27	−0.12	+0.09	−0.01
SD	0.29	0.28	0.33	0.01
Final pH				
$\bar{X}$	7.06	7.20***	7.40	7.43**
SD	0.07	0.08	0.02	0.02
Final PCO_2				
$\bar{X}$	70	60***	49.5	47.7
SD	7	8	2.2	2.8

[a] For measurements of net movement of electrolytes and water, signs indicate movement into (+) or out of (−) the lumen. Rates of net transport of electrolytes or water are expressed as micromoles or milliliters × gram wet weight of intestine^{-1} × 20 min^{-1}. The values of pH and PCO_2 (Torr) are those measured in the collected perfusion fluid; $N = 8$. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

transport during the control period are reduced to 17.8 and 16.5, respectively, but the means still do not differ significantly.

Effect of intralumenal bethanechol. Bethanechol did not affect the net movement of electrolytes and water, the pH, or the PCO_2 of the perfusate.

Discussion. The increase in the electrical negativity of the lumenal PD is similar to that observed in the dog jejunum with bethanechol (3), in the rat jejunum *in vivo* with acetylcholine (5), or pilocarpine (4), and *in vitro* with acetylcholine or neostigmine (5). Part of this change may be attributed to an enhanced net movement of Cl into the lumen (3, 4), or to a reduction in HCO_3 absorption and H secretion (4), but other unknown factors may contribute.

The changes in transport induced by bethanechol are generally similar to those caused by pilocarpine which differs from bethanechol in structure. However, whereas pilocarpine reduced the secretion of H and caused secretion of Na, K, Cl, and HCO_3, the effect of bethanechol was more selectively on Na, HCO_3, and H. This is additional evidence of a close link between the absorption of Na and the secretion of H in the rat jejunum (6).

It is difficult to estimate the contribution of reduced transit time to the changes in net ion movement in this preparation. Three findings support the view that the role of transit time was not predominant, however: (i) the changes in PD that reflect a direct effect of bethanechol on mucosal ion transport, (ii) the absence of major changes in ion transport in the ileum where contractility would also be expected to increase, and (iii) the absence of a reduction in Cl secretion, for Cl secretion should diminish with transit time.

The changes in net transport evoked by pilocarpine are quantitatively similar in the

jejunum and ileum (4), whereas the major effect of bethanechol is on the jejunum, implying that the muscarinic receptor of the ileum is less accessible or is less responsive to bethanechol. Since intralumenal bethanechol had no effect on transport, the receptor in the sensitive jejunum does not lie on the external surface of the plasma membrane. Although bethanechol might affect extraintestinal sites that could influence mucosal function through hormones or extrinsic nerves, the responsible receptors are probably located in the basolateral cell membrane or in the intrinsic nerves.

The present study shows that the systems that transport salt and water in the jejunum of the rat have the capacity to respond to the acetylcholine analogue, bethanechol. It remains to be demonstrated that this has any physiological significance.

Summary. In studies of rat jejunum, parenteral bethanechol increases the transmural PD, and decreases Na and HCO_3 absorption, the rate of H secretion, and the final PCO_2. In the ileum, it increases the final pH slightly. Bethanechol has no effect on intestinal transport when added to lumenal fluid. The changes in net transport evoked by pilocarpine are quantitatively similar in jejunum and ileum, whereas the major effect of bethanechol is on the jejunum, implying that the muscarinic receptor of the ileum is less accessible or less responsive to bethanechol.

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