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THE EFFECT OF ADRENERGIC AGENTS AND THEOPHYLLINE ON SODIUM FLUXES ACROSS THE RABBIT COLON *IN VITRO*

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Abstract—Sodium fluxes across the distal colon of the rabbit were studied *in vitro*, using $^{22}NaCl$. Sodium flux from mucosa to serosa (J_{MS}^{Na}) exceeded the flux from serosa to mucosa (J_{SM}^{Na}) resulting in net sodium movement (J_{net}^{Na}) from mucosa to serosa. Addition of an alpha adrenergic agonist (phenylephrine) increased J_{net}^{Na} by augmentation of J_{MS}^{Na}. An alpha-adrenergic antagonist (phentolamine) abolished the effect of phenylephrine. Sodium fluxes and the stimulation of J_{MS}^{Na} by phenylephrine were unaffected when calcium was omitted from the medium. Beta adrenergic agonist (isoproterenol) or theophylline did not affect J_{net}^{Na} but caused a simultaneous increase of both J_{SM}^{Na} and J_{MS}^{Na}. The role of sympathetic innervation of the colon in the regulation of sodium transport *in vivo* is discussed.

Sympathetic innervation of various visceral organs can affect the function of these organs in different ways: (1) constriction of the blood vessels supplying an organ can reduce the blood flow and thus affect function, e.g. in the kidneys or in the gastrointestinal tract; (2) through an action on smooth muscle tone and contraction in tubular organs (as the ureter, the urinary bladder or the intestine), propagation of the luminal content can be affected; (3) the possibility of a direct effect of the adrenergic transmitter on the function of the epithelial cells lining the lumen has been suggested.

Catecholamines thus have been reported to act directly on ion transport through frog skin [1], toad bladder [2] and the proximal tubules of the kidney [3]. Catecholamines have also been reported to act on ion transport in the small intestine [4,5].

We have recently studied sodium transport in the rabbit colon *in vitro* [6]. In an *in vitro* preparation of the rabbit colon mounted between two chambers (according to Ussing and Zerahn [7]), any effect on vascular or intestinal smooth muscle can be avoided, and thus a method for direct measurement of the effect on ion transport is provided. The effect of alpha-adrenergic stimulation, beta-adrenergic stimulation and theophylline in this preparation are reported in the present communication.

MATERIALS AND METHODS

Rabbits of either sex weighing 2–3 kg were used throughout. Following pentobarbital injection i.v., the distal colon was immediately removed through a midabdominal incision and was thoroughly washed in ice-cold saline. Segments of the distal colon were mounted as a membrane separating two perspex chambers, as previously described [6].

The tissues were bathed in Krebs' solution (composition: NaCl, 118 mM; KCl, 4.7 mM; $MgSO_4 \cdot 7H_2O$, 1.2 mM; $CaCl_2$, 2.5 mM; KH_2PO_4, 1.2 mM; $NaHCO_3$, 25 mM and glucose, 5g/l). A mixture of 95% O_2: 5% CO_2 was bubbled through the solution and the pH was 7.4.

Transmural fluxes of ^{22}Na from the mucosa to the serosa (M → S) and from the serosa to the mucosa (S → M), were determined by introduction of $^{22}NaCl$ (0.07 μCi/ml) into the chamber facing either the mucosal or the serosal surface of the tissue. Thirty minutes after the addition of the labelled sodium (time required to achieve a steady-state rate of radioisotope transfer), 0.5 ml samples of the solution from both serosal and mucosal chambers were taken at 5 min intervals for 40 min. ^{22}Na was then assayed in a Packard Gamma-spectrometer. Sodium flux is expressed as μmoles/cm² × hr.

The following solutions were used to study the effect of phenylephrine, isoproterenol and theophylline on sodium fluxes:

(a) Krebs' Ringer containing phenylephrine or phentolamine (Regitine) or both compounds. Each of the drugs was used at a final concentration of 5×10^{-5}M. This concentration of phenylephrine caused maximal effect and phentolamine at this concentration caused complete inhibition of the effect;

(b) Krebs' Ringer containing isoproterenol, in a final concentration of 10^{-4}M, in the presence of 0.001% ascorbic acid (in order to protect isoproterenol from oxidation);

(c) Krebs' Ringer containing theophylline, in a final concentration of 10^{-2}M. The different solutions were added either to the serosal or to the mucosal side of the mounted colon 30 min before the addition of ^{22}Na. Sodium fluxes were then determined as described above.

Chemicals. Phenylephrine and theophylline were purchased from Sigma, St. Louis, MO, U.S.A. Phentolamine was generously supplied by CIBA, Basel, Switzerland. Isoproternol was a generous gift of Assia Chemicals, Ramat Gan, Israel.

The lines in the figures were drawn according to the method of least squares.

RESULTS

(1)*Effect of phenylephrine on sodium fluxes.* Transmural fluxes of ^{22}Na through the rabbit distal colon were studied in an *in vitro* system, where a segment of the colon served as a membrane separating two compartments. As seen in Fig. 1, left side, sodium fluxes through the colon were linear throughout the duration of the experiment. Under control conditions, Na-transfer from the mucosal (M) to the serosal (S) compartment (J^{Na}_{MS}) was 1.90 μmole/cm^2 × hr, and from the serosal to the mucosal compartment (J^{Na}_{SM}) was 1.06 μmole/cm^2 × hr. Therefore, net sodium transport was observed from M to S (J^{Na}_{net}) at a rate of 0.84 μmole/cm^2 × hr.

Addition of phenylephrine (at a final concentration of 5×10^{-5}M) to the serosal side of the colon caused a significant increase of J^{Na}_{net}, as seen in Fig. 1, right side. This effect was due solely to an increase of J^{Na}_{MS}, while no effect was observed on the flux from serosa to mucosa (not shown in figure).

In the presence of phentolamine, an alpha adrenergic antagonist (final concentration 5×10^{-5}M), the effect of phenylephrine on net sodium flux was completely abolished, as seen in Fig. 1, right side. Phentolamine itself had no effect on sodium fluxes through the colon (not shown in figure).

When these experiments were repeated in a calcium-free medium, the enhancement of sodium flux by phenylephrine was unaffected. The control fluxes of sodium, J^{Na}_{MS} or J^{Na}_{SM} were no different from the respective fluxes in the calcium-containing medium.

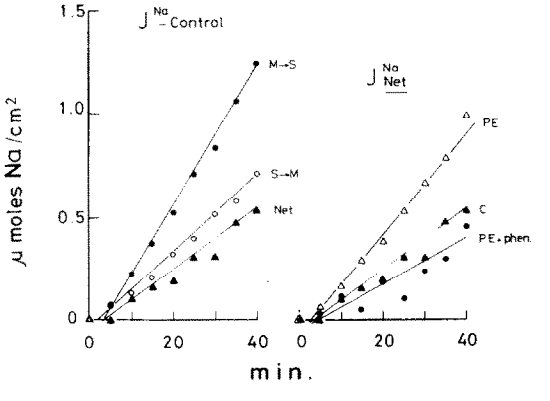

Fig. 1. Effect of alpha adrenoceptor stimulation and blockade on sodium fluxes across the rabbit colon. Left side — control experiments (N=18). M→S: sodium flux from mucosa to serosa (J^{Na}_{MS}). S→M: sodium flux from serosa to mucosa (J^{Na}_{SM}). Net: net sodium transfer from mucosa to serosa (J^{Na}_{net}). Right side — Effect of alpha adrenoceptor activation and blockade on net sodium transfer from mucosa to serosa. C: net sodium transfer from mucosa to serosa in control experiments (N=18). PE: net sodium transfer from mucosa to serosa in the presence of 5×10^{-5}M phenylephrine (N=9). PE + phen: net sodium transfer from mucosa to serosa in the presence of 5×10^{-5}M phentolamine and phenylephrine (N=9). Line for C and PE + phen not significantly different; P <0.001 for line PE.

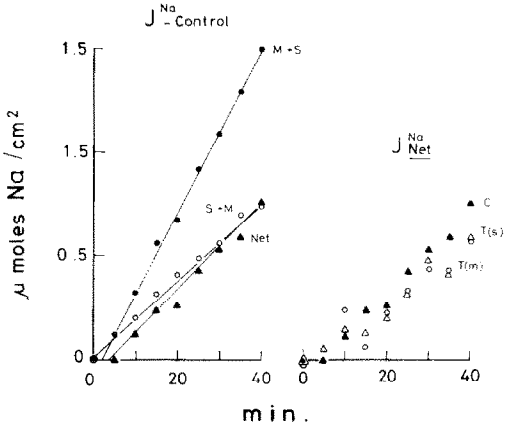

Fig. 2. Effect of theophylline on net sodium transfer from mucosa to serosa in rabbit colon. Left side — control emperiments (N=9). M→S: sodium flux from mucosa to serosa (J^{Na}_{MS}). S→M: sodium flux from serosa to mucosa (J^{Na}_{SM}). Net : net sodium transfer from mucosa to serosa (J^{Na}_{Net}). Right side — Effect of theophylline on net sodium transfer from mucosa to serosa. C: Net sodium transfer from mucosa to serosa in control experiments (N=9). T(s): Net sodium transfer from mucosa to serosa in the presence of 10^{-2}M theophylline on serosal side (N=5). T(m): Net sodium transfer from mucosa to serosa in the presence of 10^{-2}M theophylline on mucosal side (N=6). No significant difference in net Na transfer between C and T(s) or between C and T(m).

(2) *Effect of theophylline on sodium fluxes.* In contrast to phenylephrine, addition of theophylline (final concentration 10mM) to the serosal or mucosal side of the colon caused no change in J^{Na}_{net} (Fig. 2, right side). However, the lack of effect on J^{Na}_{net} was the result of simultaneous stimulation by theophylline of both J^{Na}_{MS} and J^{Na}_{SM} as seen in Fig. 3. Theophylline added to the chamber facing the serosal side had a greater effect than theophylline in the chamber facing the mucosal surface of the colon (Fig. 3).

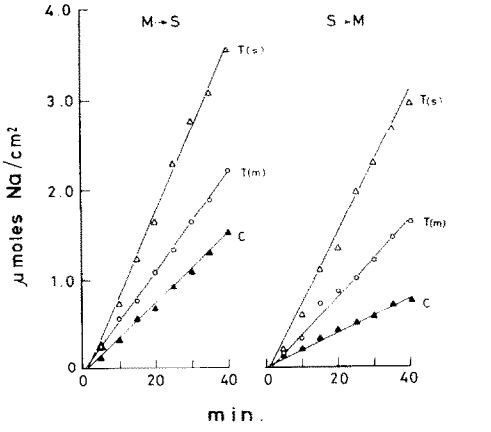

Fig. 3. Effect of theophylline on sodium fluxes across rabbit colon. Left side — sodium flux from mucosa to serosa (M→S). Right side — sodium flux from serosa to mucosa (S→M). C: control experiments (N=9). T(s): in the presence of 10^{-2}M theophylline on the serosal side (N=5). T(m): in the presence of 10^{-2}M theophylline on the mucosal side (N=6). Significance for lines T(s) and T(m), both for M→S and for S→M: P<0.001.

Addition of the beta-adrenergic agonist, isoproterenol (final concentration 10^{-4}M), to the chamber facing the serosal surface of the colon had the same effect as that shown by theophylline, i.e. simultaneous stimulation of J^{Na}_{SM} and J^{Na}_{MS} with no significant changes in net sodium flux (J^{Na}_{net}).

DISCUSSION

The results presented in this communication show that alpha-adrenergic stimulation can enhance the net transfer of sodium through the colonic mucosa. This is due mainly to an increased flux from mucosa to serosa. Since sodium absorption from the lumen depends on the activity of a sodium pump in the serosal surface of the mucosa [6], i.e. the activity of Na,K-ATPase, a possible mechanism could be that alpha-adrenergic agonists stimulate the Na,K-ATPase. Such activation has been reported in nerve cells (adrenal medulla, Gutman and Boonyaviroj [8]; brain, Lee and Phillis [9]). Stimulation of the sodium pump to produce hyperpolarization has also been described in skeletal muscle [10]. A similar mechanism thus may be envisaged for the rabbit colon.

Furthermore, adrenergic stimulation of sodium transport transcellularly, i.e. across an epithelial cell separating two compartments, has been described in several epithelia: in frog skin [1], toad bladder [2], the proximal tubules of the kidney [11], [12] and reviewed recently by Gottschalk [3] and in the small intestine [4,5]. Adrenergic stimulation of sodium transport may therefore represent a general phenomenon, particularly important in epithelia.

Recently several studies have pointed to the possible involvement of intracellular calcium in changes of permeability to sodium and potassium [13–15]. We have therefore repeated the study of alpha-adrenergic stimulation in calcium-free media. However, no significant change was observed either in the control sodium fluxes or in the effect of phenylephrine on the fluxes in calcium-free medium. However, the role of intracellular calcium cannot be completely dismissed because changes of free intracellular calcium could still occur due to intracellular storage organelles.

cAMP has been shown to cause loss of fluid into the lumen in the small intestine [16]. The effect of theophylline and of salbutamol on the colon shows a qualitative resemblance but no net loss of fluid into the lumen, in contrast to the ileum. Both fluxes, through the mucosa and through the serosa, were enhanced to the same extent (Fig. 3). This could be due to increased permeability to sodium caused by the drug with no effect on the sodium pump. It would seem plausible to assume that the change in permeability occurred mainly in the serosal surface: an increase of the mucosal (luminal) permeability would result in stimulation of the serosal sodium pump due to the rise of intracellular sodium concentration and should therefore increase net sodium flux from mucosa to serosa. Such a combination, i.e. increased luminal permeability coupled with stimulation of the sodium pump, follows administration of mineralocorticoids.

The finding that serosal application of theophylline was more effective than mucosal application (Fig. 3) may suggest that the serosal membrane is more permeable to theophylline than the mucosal (luminal) membrane. Application of 7.5 mM cAMP has been reported to produce in the rabbit colon an effect similar to that of theophylline reported here, i.e. increase of J^{Na}_{MS} and J^{Na}_{SM} to the same degree [17].

In the present set of experiments we have studied fluxes across the isolated colonic mucosa *in vitro*. However, in assessing the *in vivo* net effect of adrenergic stimulation, it is important to recall its vascular action in the intestine, i.e. vasoconstriction. This would decrease perfusion of the colon and thus reduce transfer of fluid from the intestine to the circulation. Possibly the stimulation of sodium net absorption through activation of alpha receptors could counteract the danger of fluid loss into the lumen under these conditions.

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