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SODIUM TRANSPORT BY ISOLATED BULLFROG SMALL INTESTINE

EFFECT OF PROSTAGLANDIN E_1

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Summary

Addition of 446 μM prostaglandin E_1 (PGE_1) to the serosal medium of isolated short-circuited bullfrog small intestine elicited small increases in transmural potential difference and short-circuit current while addition of PGE_1 to the mucosal medium caused no change in the electrical parameters. Addition of 100 μM indomethacin to the mucosal medium inhibited both potential difference and short-circuit current with a resultant increase in steady-state tissue resistance. In the presence of mucosal 100 μM indomethacin, serosal 60 μM PGE_1 markedly stimulated transmural potential difference and short-circuit current with a resultant decrease in steady-state tissue resistance. Serosal arachidonic acid (330 μM) stimulated transmural potential difference and short-circuit current and this effect was abolished by the addition of 100 μM indomethacin to the mucosal medium. Serosal 60 μM PGE_1 only stimulated the M (mucosa) → S (serosa) unidirectional flux of sodium. These results strongly suggest that the PGE_1 action is mediated either via a series of metabolic reactions which possibly increase the permeability of the mucosal membrane to sodium or via direct stimulation of rheogenic sodium pump activity.

Introduction

Pierce et al. [1] demonstrated that infusion of prostaglandins (PGE_1 and $PGF_{2\alpha}$) and theophylline into the mesenteric artery resulted in a cholera-like secretion of water and electrolytes by in vivo canine jejunum; the effects of the two agents were synergistic. These effects are unlikely to be secondary to their hemodynamic activity since PGE_1 and $PGF_{2\alpha}$ have very similar effects on fluid and electrolyte transport in doses which have diametrically opposed effects on splancnic blood flow [2]. A primary effect on the mucosa is suggested by experiments on the isolated rabbit ileal mucosa in which prostaglandins applied

to the serosal surface stimulated active chloride secretion and inhibited sodium absorption [3]. The effects of prostaglandins on ion transport in rabbit and human isolated ileal mucosa are similar to those produced by cholera exterotoxin, theophylline and dibutyryl cyclic adenosine monophosphate [4,5]. The observation that prostaglandins and cholera exterotoxin markedly increase gut mucosal adenyl cyclase activity [6] suggests that both act by increasing the intracellular concentration of cyclic adenosine monophosphate which in turn stimulates an increase in fluid and electrolyte secretion from the intestinal mucosa.

Similar models of sodium transport across the intestine have been proposed by Nellans et al. [7] and Quay and Armstrong [8]. It has been suggested by Nellans et al. [7] that cyclic adenosine monophosphate exerts its stimulation of ion secretion through a NaCl-coupled transport mechanism located in the mucosal membrane of the epithelial absorptive cell. However, as suggested by the above authors [7,8], at least two other routes for transcellular movement of Na^+ exists. Hence, this investigation was undertaken to study the effect of prostaglandin E_1 (PGE_1) on the Cl^--independent Na^+ transport system across the intestine.

Methods and Materials

Flat sheets of small intestine from adult bullfrog (*Rana catesbeiana*) were prepared and mounted between the two halves of a lucite chamber similar to that described by Quay and Armstrong [8]. The chamber used was a modification of one used by Zadunaisky [9] in studies on isolated corneal tissue, with a 0.33 cm^2 circular aperture between the two halves. The fluid volume in each half chamber was 5 ml. The mucosal and serosal media were circulated and gassed continuously with 100% O_2 as described by Ussing and Zerahn [10].

The tissue was mounted between identical phosphate-buffered Cl^--free Na_2SO_4 Ringer solution made isosmotic with mannitol, of the type described by Adrian [11]. The Ringer had a total osmolality of 230 mosM and a pH of 7.2 at 25°C.

Transmural potential difference (PD) and short-circuit current (SCC) were measured by methods similar to those employed by Schultz and Zalusky [12] with the modifications described by Quay and Armstrong [8]. A voltage clamp device, with automatic compensation for the voltage drop due to the resistance of the bathing solution [13], was used to maintain short-circuit conditions.

The experiments with the Na_2SO_4 medium were performed as follows. After the tissue was excised and mounted, SCC and PD were recorded every 5 min until an electrical steady state was reached. This usually required from 30 to 60 min. When sufficient measurements had been made to establish the steady-state SCC and PD, the activator or inhibitor to be tested was added, either by removing the control Ringer solution from the chamber and replacing it with one containing the activator or inhibitor at the concentration desired, or by directly adding a small volume (usually 10—50 μl) of a stock solution of the substance to one or both chamber compartments. Following this above procedure, measurement of SCC and PD were continued as before.

Using ^{22}Na (New England Nuclear) unidirectional mucosa (M) to serosa (S)

and S to M fluxes of Na^+ were determined on paired tissue when their respective short-circuit currents were comparable in magnitude. In these experiments the tissue was allowed to equilibrate for 30—90 min in non-radioactive Ringer solution. At this electrical steady-state time a trace amount of isotope was added directly to the chamber. Thereafter, at timed intervals of approx. 20 min, 0.1-ml samples of solution were removed from the initially unlabeled half-chamber for counting. The samples were counted in a two channel Packard Series 5000 autogamma spectrometer. From the results obtained, M to S (J_{MS}) and S to M (J_{SM}) Na^+ fluxes were computed as described by Quay and Armstrong [8].

All reagents were analytical reagent grade and all solutions were made up in deionized water with the exception of PGE_1 which was initially solubilized in 95% ethyl alcohol.

Results

Effect of PGE_1 on PD and SCC

In oxygenated substrate-free Na_2SO_4 Ringer solution, addition of PGE_1 (446 μM) to the serosal medium elicited small increases in PD and SCC. The onset of these changes was relatively slow. They were sustained sometimes for long periods of time (1—3 h), and were abolished by rinsing and replacing the serosal compartment with fresh PGE_1-free Ringer solution. The magnitude of these electrical changes was always quite small. In some experiments we did not observe a change in PD or SCC following administration of 446 μM, 1.49 or 2.97 mM PGE_1 to the serosal medium. Mucosal PGE_1 (446 μM) had no effect on PD or SCC, and no effect on these characteristics was observed following rinsing and refilling the mucosal compartment with fresh PGE_1-free Ringer solution.

The effects of metabolic inhibitors and of ouabain on the increase in PD and SCC elicited by serosal PGE_1 were also studied. Representative examples of these experiments are illustrated in Figs. 1—3. Fig. 1 shows that PGE_1 markedly stimulated PD and SCC in the presence of mucosal indomethacin. The time required for the onset of inhibition by indomethacin was 1—5 min and the inhibition was reproducible, but rarely complete. Indomethacin increased steady-state tissue resistance (PD/SCC), i.e., there was a greater inhibition

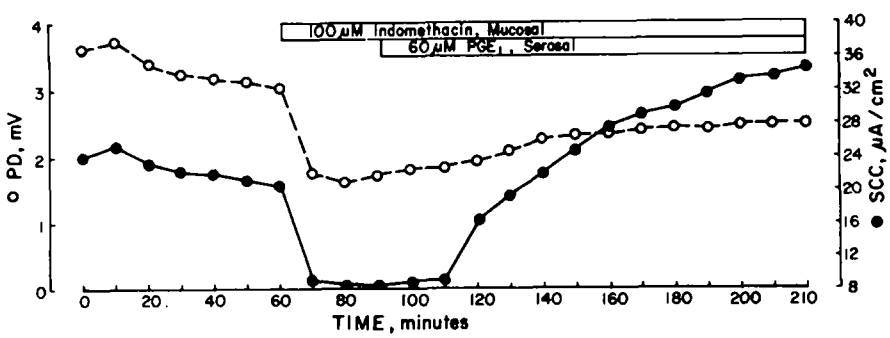

Fig. 1. Stimulation of SCC (short-circuit current) and PD (potential difference) by 60 μM serosal PGE_1 following inhibition by 100 μM indomethacin.

of SCC relative to that of PD. However, PGE_1 greatly reduced steady-state tissue resistance (even below initial control values) in the presence of indomethacin. The average percentage reduction ($N = 10$) in tissue resistance from that of control tissue (indomethacin-treated) was 30 ± 12 and this was significantly greater than zero ($P < 0.01$). The time for onset of stimulation of PD and SCC by PGE_1 was much less in the presence of indomethacin than in its absence. Rinsing and refilling the serosal compartment with fresh PGE_1-free Ringer partially decreased PD and SCC. However, the addition of 60 μM PGE_1 to the mucosal bathing medium in the presence of 100 μM mucosal indomethacin caused no change in PD or SCC. Since PGE_1 gave reproducible, magnified increases in the electrical parameters, PD and SCC, in the presence of mucosal indomethacin, indomethacin was commonly employed in all subsequent experiments using PGE_1.

Fig. 2 shows that 2,4-dinitrophenol inhibited PD and SCC in the presence of serosal PGE_1 and mucosal indomethacin. Conversely, addition of serosal PGE_1 (60 μM) following inhibition of PD and SCC by mucosal indomethacin (100 μM) and 2,4-dinitrophenol (1 mM) respectively, elicited little or no increase in these parameters.

Fig. 3 shows that serosal ouabain inhibited SCC and PD in the presence of serosal PGE_1 and mucosal indomethacin. The onset of inhibition of ouabain (5—20 min) and the time course of inhibition by this compound were essentially the same whether PGE_1 and indomethacin were present or not. Ouabain inhibited both the PGE_1-induced SCC and PD and the endogenous SCC and PD. Conversely, addition of 60 μM PGE_1 to the serosal solution following inhibition of PD and SCC by mucosal 100 μM indomethacin and serosal 2 mM ouabain, respectively, elicited little or no increase in these electrical characteristics.

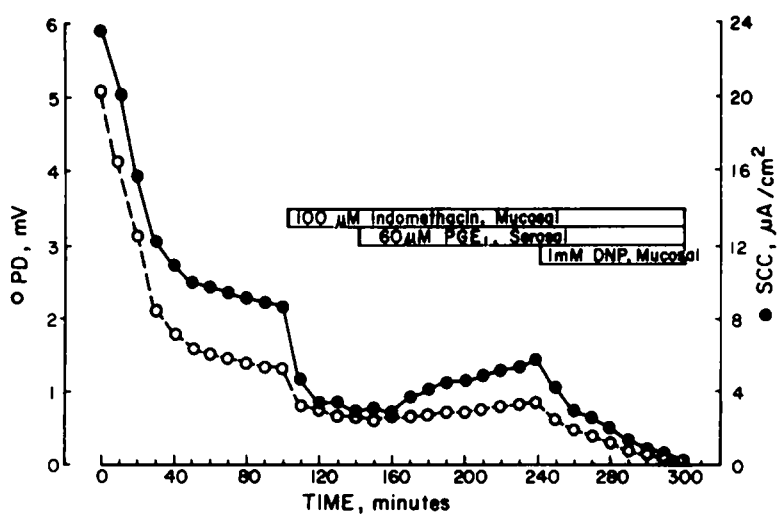

Fig. 2. Effect of 1 mM dinitrophenol (DNP) on SCC and PD in the presence of serosal PGE_1 (60 μM) and indomethacin.

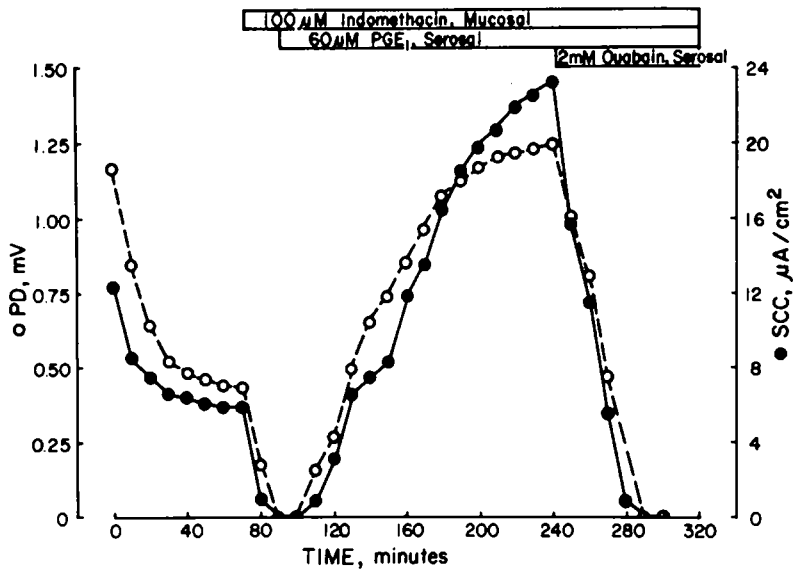

Fig. 3. Stimulation of SCC and PD by 60 μM PGE_1 in the presence of 100 μM indomethacin and inhibition of PGE_1-elicited SCC and PD by 2 mM serosal ouabain.

Differential effects of actively transported sugars and amino acids, adenosine triphosphate (ATP) and PGE_1 on PD and SCC

In some respects the PGE_1-induced increases in PD and SCC reported here resemble the increases in these electrical characteristics observed in the presence of actively transported sugars and amino acids [14] and ATP [15]. In both cases these increases are well sustained and are reversed by rinsing and replacing the solute-containing compartment with fresh solute-free medium. These points of resemblance raised the possibility that the enhancing effect of PGE_1 on PD and SCC might share a common mechanism with the effects produced by actively transported sugars or amino acids or ATP. In an attempt to obtain more definitive evidence concerning possible interrelationships between the effects of actively transported organic solutes, ATP and PGE_1, a further series of experiments was performed. Representative examples of these experiments are illustrated in Figs. 4 and 5.

First, it was found that following virtually maximal stimulation of PD and SCC by 12 mM mucosal glucose in the presence of indomethacin, a further increase in these parameters could be obtained by addition of 60 μM PGE_1 to the mucosal medium. It was further found that following virtually maximal stimulation of PD and SCC by mucosal D-glucose in the presence of mucosal indomethacin, serosal PGE_1 stimulated the electrical parameters after mucosal phlorizin inhibited the glucose-induced increased electrical characteristics (Fig. 4). It was also found that after stimulation of SCC and PD by serosal PGE_1 in the presence of mucosal indomethacin, 20 mM L-alanine caused an immediate, additional increase of the electrical parameters (Fig. 5). A similar result was found with the addition of 5 mM ATP to the mucosal solution after

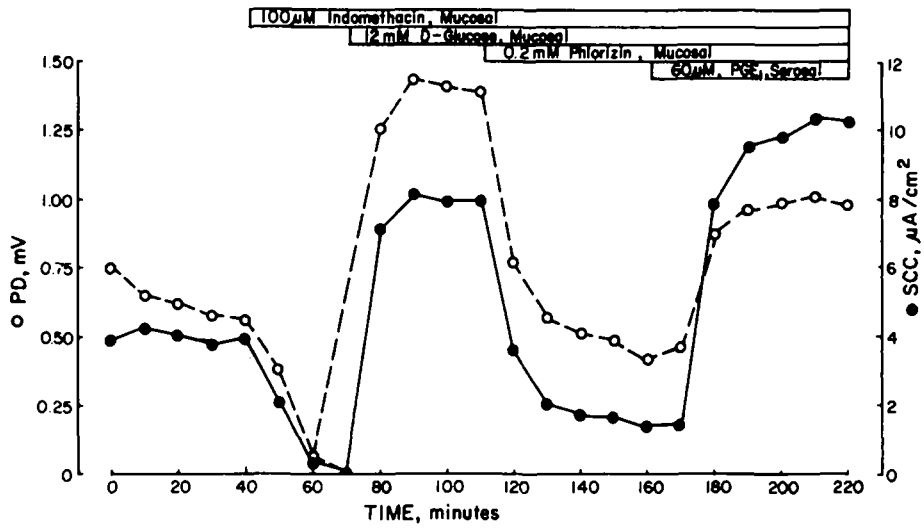

Fig. 4. Differentiation between effects of glucose and PGE_1 on SCC and PD.

serosal 60 μM PGE_1 stimulated SCC and PD in the presence of mucosal 100 μM indomethacin.

Response of PD and SCC to a precursor of PGE_1

Rapid sustained increases in PD and SCC were obtained following addition of arachidonic acid to the serosal medium (Fig. 6). The time course for onset of stimulation was more rapid than that of PGE_1, however, the reduction in steady-state tissue resistance caused by arachidonic acid was approximately the same as that caused by PGE_1. Fig. 6 also shows that the arachidonic acid-induced increases in PD and SCC can be inhibited by the addition of indomethacin to the mucosal medium. Conversely, arachidonic acid added to the

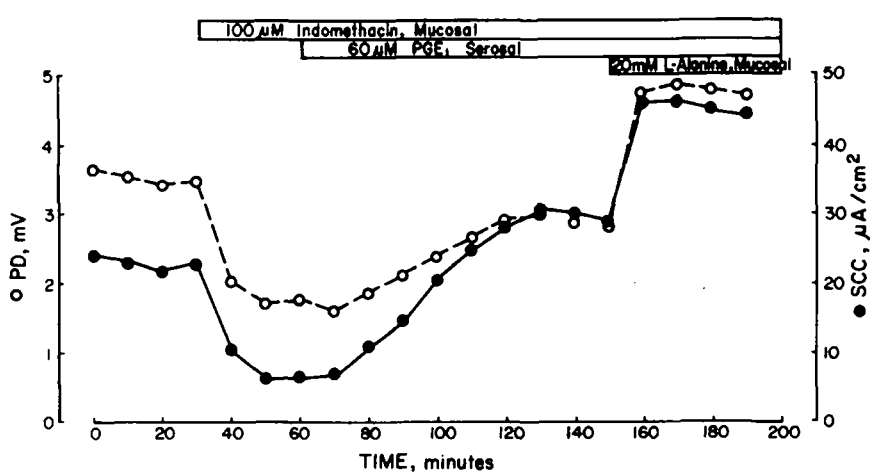

Fig. 5. Additive effects of 60 μM serosal PGE_1 and 20 mM mucosal L-alanine.

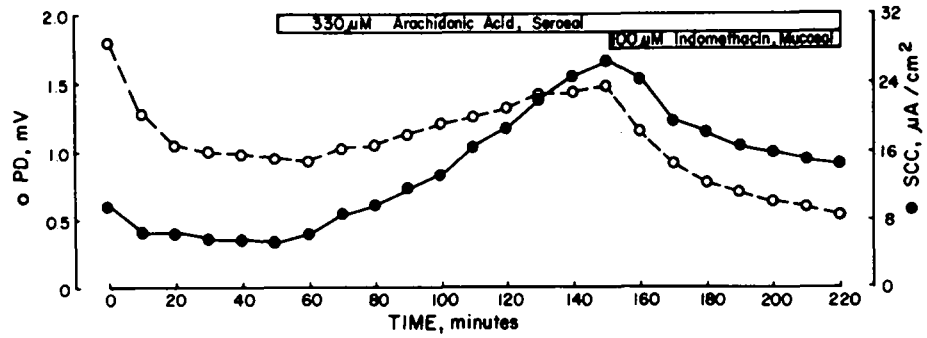

Fig. 6. A typical experiment demonstrating the effect of mucosal 100 μM indomethacin on SCC and PD stimulated by serosal 300 μM arachidonic acid.

serosal medium in the presence of indomethacin in the mucosal medium elicited no increase in PD or SCC. Also, there was no change in steady-state tissue resistance by serosal arachidonic acid in the presence of mucosal indomethacin.

Addition of 500 μM linoleic acid to either the mucosal or serosal media caused no changes in PD or SCC.

Dose vs. response of SCC to PGE_1

Fig. 7 shows a dose vs. response relationship between concentration of PGE_1 administered to the serosal medium (in the presence of indomethacin) and increase in SCC. As is seen, SCC increased curvilinearly with increasing concentrations of PGE_1 added to the serosal medium.

Response of PD and SCC to cyclic adenosine monophosphate and theophylline

Addition of 5 mM cyclic adenosine monophosphate (cyclic AMP) or 7.5 mM theophylline to the serosal medium evoked increases in PD and SCC similar to those evoked by PGE_1. However, after virtually maximal stimulation of PD and SCC by PGE_1 in the presence of indomethacin, addition of 7.5 mM theophylline to the serosal medium caused no further increase in the measured electrical characteristics.

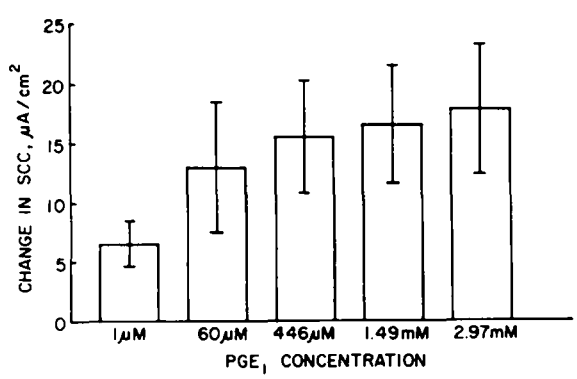

Fig. 7. Dose vs. response relationship between increasing serosal concentrations of PGE_1 and change in SCC in indomethacin-treated gut. Each bar represents the mean of 4—15 experiments. Vertical deviations represent the standard error of the mean.

TABLE I

SODIUM FLUXES IN SODIUM SULFATE AMPHIBIAN RINGER

Average values ± S.E.M. are given for the number of experiments shown in parentheses. Results are expressed in nequiv./cm^2 per min.

	J_{MS}	J_{SM}	J_{MS}^{NET}	Short-circuit current
Before PGE_1 addition	43.4 ± 3.8 (6)	46.9 ± 4.8 (6)	−3.5 ± 4.1	0.8 ± 0.3
After PGE_1 addition	59.3 ± 4.3 (6)	49.3 ± 5.3 (6)	10.0 ± 4.9	9.2 ± 1.6

Effect of PGE_1 on Na^+ fluxes

The increases in PD and SCC elicited by serosal PGE_1 are indicative of an enhanced net mucosal to serosal transfer of positive charge in the presence of this compound. The increases induced in these characteristics by actively transported sugars, amino acids and ATP have been accounted for in terms of an increased net mucosal to serosal movement of Na^+ [14,15]. Therefore, in the present investigation the effect of serosal PGE_1, in the presence of indomethacin, on the unidirectional mucosal to serosal (J_{MS}) and serosal to mucosal (J_{SM}) fluxes of Na^+ was investigated directly by isotopic methods.

In all of the experiments the steady-state SCC was very low before PGE_1 addition. Correspondingly, no significant difference between J_{MS} and J_{SM} for the group was observed during this time (Table I). Following addition of PGE_1, the mean J_{MS} significantly increased ($P < 0.05$) while the mean J_{SM} did not significantly change. The mean J_{MS}^{NET} of Na^+ after PGE_1 addition was not significantly different ($P > 0.35$) from the corresponding SCC during this time. Following PGE_1 addition, the mean J_{MS}^{NET} of Na^+ significantly increased ($P < 0.05$).

Discussion

Addition of PGE_1 to the serosal compartment bathing an isolated sheet of bullfrog small intestine elicited small increases on the measured PD or SCC while addition of PGE_1 at the same concentration to the mucosal compartment caused no change in the electrical characteristics. However, in the presence of indomethacin, an inhibitor of prostaglandin synthetase, where endogenous prostaglandin synthesis and release presumably was reduced, PGE_1 added to the serosal bathing medium markedly stimulated both SCC and PD (Fig. 1). These observations suggest that the mechanisms through which PGE_1 stimulated PD and SCC is near-saturated, in the absence of indomethacin, by endogenous prostaglandins. In the reduction or absence of endogenous prostaglandins by indomethacin treatment, exogenous PGE_1 can exert its stimulatory effect because of the lack of competition with its endogenous counterpart. Indomethacin administered to the intestine increases tissue resistance suggesting the possibility that endogenous levels of prostaglandins modulate a level of tissue resistance. Strengthening this notion is the observa-

tion that transmural tissue resistance (PD/SCC) was significantly reduced in the presence of PGE_1 (Fig. 1).

It appears that the site of PGE_1 action is localized in or around the lateral-serosal membranes of the epithelial absorptive cells in the bullfrog small intestine, for addition of PGE_1 to the mucosal solution in the presence of a prostaglandin synthetase inhibitor (e.g. indomethacin) caused no change in the base-line electrical characteristics. The serosal PGE_1-stimulated increase in SCC, PD and transmural tissue conductance could be mediated either via (1) a series of metabolic reactions because of: (a) the delay before onset of PGE_1 action and (b) the long time course of action before maximal effects were produced (Fig. 1) or via (2) a direct stimulation of a rheogenic Na^+ pump located in the lateral-serosal membrane. The slow time course of PGE_1 effects on PD and SCC could be diffusion delay because of the adventitious tissue separating the lateral-serosal membrane from the serosal medium.

The fact that there were saturable electrical effects with regards to the increasing concentrations of PGE_1 used suggests that the mechanism for inducing these changes is specific and limited in capacity (Fig. 7).

Although the concentrations of prostaglandins at the level of the bullfrog intestinal absorptive cell is not known, the concentrations of prostaglandin E_1 used in the present study were comparable to those used in other in vitro epithelial studies [5,16,17].

Arachidonic acid, a precursor of prostaglandins, produces the same effects as seen with PGE_1 (Fig. 6), that is, an increase in both SCC and PD with a decrease in transmural resistance. Indomethacin inhibits the arachidonic acid-induced increase in SCC and PD strongly suggesting that indomethacin specifically inhibits prostaglandin synthetase and that arachidonic acid needs to be converted to prostaglandin before it can induce increases in SCC and PD (Fig. 7). Supporting this contention is the observation that addition of a non-prostaglandin precursor (linoleic acid) to the serosal or mucosal bathing medium caused no change in PD or SCC.

The observation that 1 mM dinitrophenol inhibits the PGE_1-induced increase in SCC and PD suggests that the PGE_1 effect is dependent upon intact aerobic metabolic pathways (Fig. 2). The observation that ouabain also inhibits the PGE_1-induced increases in both SCC and PD suggests that PGE_1 action on the electrical characteristics is dependent upon cellular ATP and its consequent hydrolysis by a ouabain sensitive ($Na^+ + K^+$)-dependent ATPase (Fig. 3).

Several points argue against the supposition that the PGE_1-induced increases in SCC and PD act through a mechanism similar to those increases in SCC and PD induced by actively transported sugars and amino acids or those produced by ATP. First, those induced increases in SCC and PD by D-glucose, L-alanine or ATP are obtained only when these solutes are placed in the mucosal bathing medium [14,15] while the PGE_1-induced increases in PD and SCC are obtained only when the prostaglandin is placed in the serosal bathing medium. Second, the D-glucose, L-alanine and ATP-induced increases in PD and SCC are very rapid (i.e., within seconds) whereas the PGE_1-induced increases takes a much longer time (Fig. 1). Third, the induced electrical effects of D-glucose, L-alanine or ATP are additive with the induced electrical effects of PGE_1 (Figs. 4 and 5).

Quay and Armstrong [8] have provided definitive evidence that the baseline SCC in bullfrog intestine bathed in a sulfate Ringer is a Na^+-carrying current, and they have proposed a model for transcellular Na^+ transport across the bullfrog small intestine. The chloride, organic solute-independent Na^+ transport system in the mucosal membrane of their model was left undefined as to the nature of possible control or modulation of this passive transport process. Prostaglandins enhance adenyl cyclase activity [6] and increase cyclic AMP levels in the intestinal mucosa [18]. It is possible that the PGE_1 stimulates Na^+ absorption in a Cl^--free Ringer solution by stimulating intestinal mucosal adenyl cyclase activity. This supposition is supported by the observation that serosal theophylline has no effect on the electrical characteristics stimulated by PGE_1, but by itself, theophylline stimulates PD and SCC in a manner similar to that of PGE_1. Since the receptor for adenyl cyclase has been deduced to be localized, on the serosal rather than the mucosal border of several epithelia [19,20], it is feasible to presume that PGE_1 would be more accessible to adenyl cyclase when it was placed in the serosal bathing medium. Hence, the reason for stimulation of the electrical parameters when PGE_1 was placed in the serosal bathing medium, and not in the mucosal bathing medium. This increased enzyme activity would lead to increased intracellular concentrations of cyclic AMP which would be the final mediator for increasing Na^+ permeability of the mucosal membrane. The increased intracellular Na^+ concentrations would then stimulate greater sodium pump activity which would be reflected in an increase in the unidirectional J_{MS} of Na^+ and SCC, which is shown in our data (Fig. 1 and Table I). PGE_1 stimulates only the J_{MS} of sodium and not the J_{SM} of sodium, therefore, it can only effect its stimulation by acting directly on the Na^+ pump in the serosal membrane [8] or by increasing the Na^+ permeability of the mucosal membrane. Since there is a significant reduction in tissue resistance by the action of PGE_1, one cannot rule out a direct action on the Na^+ pump, since an alteration of pump resistance would bring about the same effect. If PGE_1 directly stimulates a rheogenic Na^+ pump located in the lateral serosal membrane, this could lower intracellular Na^+ concentration and therefore, indirectly could increase the permeability of the mucosal membrane to Na^+ as suggested by Biber [21] and Lewis and Diamond [22]. Our data however, does not allow us to distinguish between the alternatives of PGE_1 acting through adenyl cyclase or by its direct action on a rheogenic Na^+ pump.

Acknowledgments

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