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Peptides increase anion conductance of canine trachea: an effect on tight junctions

P.K. Rangachari and D. McWade

Intestinal Disease Research Unit, Department of Medicine, McMaster University, Hamilton, Ontario L8N 3Z5 (Canada)

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The ionic basis for the rapid reduction in potential difference (dip) produced on luminal addition of substance P and related peptides was analysed by altering the electromotive force and chemical gradients across the isolated, canine tracheal epithelium. The dip could be exaggerated, minimised or reversed by increasing, decreasing or reversing the basal potential difference, and the intercept of the line relating the two was close to zero when the Cl^- compositions of the two bathing solutions were identical. Luminal Cl^- replacement by a non-permeant anion (isethionate) attenuated the dip which was, however, exaggerated by a permeant anion (nitrate). Replacement of serosal Na^+ or luminal HCO_3^- had no significant effect on the magnitude of the dip. The tachykinins exhibited cross-tachyphylaxis with each other, indicating a common receptor. Bradykinin, a structurally unrelated peptide, also produced dips upon luminal addition, but showed no cross-tachyphylaxis with the tachykinins. Again, a linear relation between basal potential difference and the dip elicited by bradykinin was observed. Based on current awareness of the bioelectric properties of the canine tracheal epithelium, we suggest that these peptides modulate paracellular anion permeabilities.

A current paradigm relates transfer of ions across epithelial sheets to two routes viz.: cellular and paracellular. Such transfers result in epithelial preparations developing transmucosal potential differences while set up in vitro. Alterations in the potential differences thus developed can be conveniently analysed in terms of the modulation of either one or both routes of permeation [1]. Although a number of transmitters and hormone have been shown to modulate transcellular ion movements, relatively few modulators of the paracellular pathway have been described; these include, among others, cyclic AMP, Ca^{2+}, carbachol, pancreozymins, and prostaglandins [2–6]. Luminal addition of several peptides, (substance P, other tachykinins, bradykinin) produce marked effects on the isolated canine tracheal epithelium, a well studied model for the airway mucosa [7–11]. Our studies reported here suggest that these too could act as modulators of the paracellular path.

We showed earlier that the addition of substance P and related tachykinins to the bathing solutions produced marked changes in transepithelial potential differences and resistances across the isolated canine trachea [10]. Luminal addition elicited a prompt, rapid, transient reduction in potential difference which we called the 'dip', followed by a rise, both phases associated with decreases in resistance. Serosal addition, however, produced only an increase in potential difference and a decrease in resistance, but after a substantially longer lag. The rapidity of the response

Correspondence: Dr. P.K. Rangachari, Intestinal Disease Research Unit, Department of Medicine, McMaster University, Hamilton, Ontario, L8N 3Z5 Canada.

elicited on luminal addition suggested that luminal receptors for tachykinins were present.

The dip being the more dramatic of the events noted, we attempted to analyse the underlying ionic mechanisms, and tested the hypothesis that the dip could reflect either an anion moving from lumen to serosa, a cation from serosa to lumen, or a combination of both. Using ion substitutions, we show here that the dip represents the opening of an anion-selective channel on the luminal side of the epithelium.

The isolated epithelium was obtained from dogs killed with sodium pentobarbital (100 mg/kg) and set up in conventional Ussing chambers for recording transmucosal potential differences and resistances. Bathing solutions on both sides were identical (composition mM): NaCl 116, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 22, NaH_2PO_4 1.2, glucose 10. The solutions were bubbled continuously with 95% O_2/5% CO_2 maintaining a pH of 7.3–7.4 at 37°C. An airlift system (MRA Corporation) was used for circulating the fluids. Transmucosal potential differences were recorded with 3 M agar-KCl bridges connected to a high impedance millivoltmeter (Iowa Bioengineering Dual Voltage Clamp) and resistances were measured with periodic pulses of 50 μA currents passed across the tissue (for details see Ref. 10). The rapid change in potential difference produced by unit current was used as a measure of transmucosal resistance. The current voltage relations in this tissue are linear in the range of 50–100 μA. In some experiments, tissues were clamped at various transmucosal potentials by passing currents across the tissue. Under basal conditions (i.e. in the absence of added peptides), clamping the potential differences to values higher or lower than baseline potential differences did not alter resistance.

Cl^- replacements were carried out using $NaNO_3$ or sodium isethionate for NaCl and acetate salts for K^+, Ca^{2+} and Mg^{2+}. Na^+ free solutions were prepared using either choline chloride or *N*-methyl-D-glucamine (titrated to neutral pH with HCl) and choline bicarbonate as replacement for $NaHCO_3$. In the bicarbonate replacement studies, Hepes buffer was used, titrated and maintained at pH 7.4.

Peptides (Penninsula) were made up in 0.01 M acetic acid, frozen in small aliquots, and were added directly to the luminal solution to give final concentrations of 10^{-7}–10^{-6} M (optimal for observing dips). Trasylol® (200 units per chamber) was added to prevent degradation. Following each peptide addition, tissues were extensively washed and an hour's recovery period allowed to avoid tachyphylaxis.

The magnitude of the dip elicited by a given concentration of substance P bore a direct linear relation to the pre-existing potential difference. The dip could be artificially exaggerated, or minimised by clamping the potential difference at various levels, prior to the addition of the peptide. Reversing the normally oriented potential difference (lumen made positive) could 'reverse' the dip, which then appeared as a notch in the trace, since the rise was not abolished. The linear relationship is clearly shown with the line passing

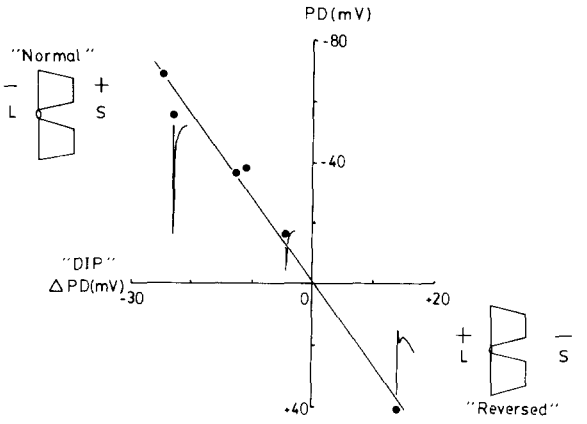

Fig. 1. The linear relation between the magnitude of the dip and the basal potential difference. In the insets, the tracheal epithelium is shown diagrammatically to indicate the orientation of the basal potential difference. In the upper left quadrant, results using a tissue with a normally oriented potential difference (i.e. lumen negative). The luminal addition of substance P (10^{-6} M) produces a sharp dip, tending to reduce to potential difference (bringing it closer to zero). The lower right quadrant, demonstrates a 'reversed' dip, the basal potential difference of the tissue having been artificially inverted (lumen made positive). Here too, the dip tends to bring the potential difference closer to zero. The results shown here are from a single experiment. The Y intercept was 0.162, $r^2 = 0.98$. In seven experiments, the Y intercept was 1.76 ± 0.56 (mean $\pm$ S.E.), and r^2 was 0.94 ± 0.027 (mean $\pm$ S.E.). Because of this relationship, tissues were clamped to control potential difference values when comparing the magnitude of the dip under trial conditions which altered basal potential difference.

close to the origin in Fig. 1. A simple explanation is that the dip represented the transient opening of a passive ionic channel in the apical membrane, the direction of the dip representing the net movement of the ion(s) driven solely by the existing electrochemical gradients, the concentrations of the ions bathing both surfaces being substantially the same. The dip could thus represent the movement of a cation from serosa to lumen, an anion from lumen to serosa or a combination of both.

Total replacement of serosal Na^+ (the major cation present) with either choline or *N*-methyl-D-glucamine did not significantly alter the dip (control 9.4 ± 1.5 mV; Na^+-free 9.5 ± 2.3 mV, in four experiments; mean $\pm$ S.E.). Total Cl^- replacement by other anions did, however, markedly alter the dip, which was exaggerated by luminal NO_3^-, a more permeant anion, and attenuated by isethionate, a non-permeant one [11–13]. In six experiments, the following values were noted (mean $\pm$ S.E.): Cl^- (control) 9.9 ± 1.5 mV; NO_3^- 18.9 ± 2.9 mV; isethionate 2.9 ± 1.0 mV, these being significantly different from the Cl^- control ($P < 0.05$). Thus the dip clearly suggests the opening of an anion selective channel on the apical surface in response to the peptide. Replacement of luminal HCO_3^- with Hepes buffer did not significantly alter the magnitude of the response (control 6.8 ± 2.0 mV; HCO_3^--free 6.2 ± 2.4 mV in four experiments; mean $\pm$ S.E.). Although Na^+ removal did not alter the dip, it was possible that an increase in apical K^+ conductance could be involved, leading to rapid depolarization and cell shrinkage. This shrinkage could in turn increase shunt permeability. However, the dips observed were not altered, either by mucosal $BaCl_2$ (10 mM) or by high K^+ (120 mM) in the luminal bathing solutions.

The potential difference developed across the isolated canine tracheal epithelium depends on the functioning of a Na^+-K^+-$2Cl^-$ symport in conjunction with the ubiquitous Na^+/K^+ pump that brings Cl^- into the cell, its exit across the apical membrane being conductive [14]. This being so, an increase in anion permeability of the apical membranes of the epithelial cell would lead to Cl^- exit from the cell, in the direction of a favourable electrochemical gradient. This would serve to increase rather than decrease the basal potential difference. If, however, the increase in Cl^- permeability occurred in the paracellular pathway (be it the tight junctions or the lateral intercellular spaces) the net result would be a shunting of the pre-existing potential difference, and the direction of this change would depend solely on the electromotive forces and chemical gradients for the ions involved. Our results are consistent with such an interpretation, and under short-circuit conditions, luminal addition of substance P produced marked and consistent increases, but not decreases in short-circuit current. The increases in current reflect the cellular component, seen on the open-circuited epithelium, as the rise in potential difference.

Dips similar to these seen with substance P have been seen with other tachykinins that share a Phe-X-Gly-Leu-Met-NH_2 sequence. Cross-tachy-

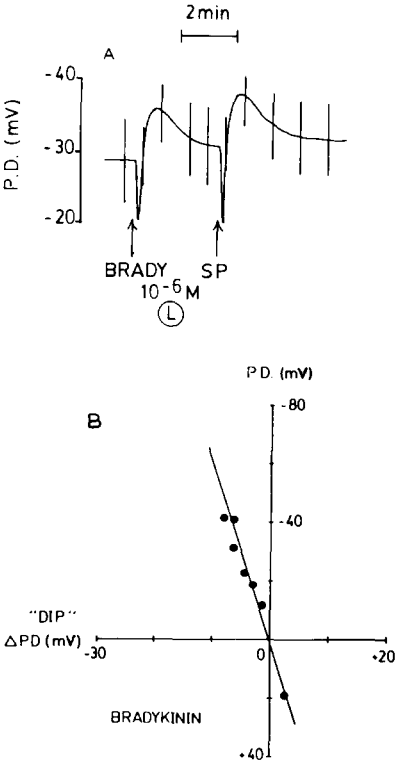

Fig. 2. (A) The response of the tracheal epithelium to luminal additions of substance P (SP) and bradykinin. No cross-tachyphylaxis is observed. (B) With bradykinin, the relationship between the basal potential difference and the magnitude of the dip is also linear, with a *Y* intercept of 0.176, and r^2 of 0.980 (single experiment).

phylaxis between these peptides suggest a common receptor, and the analysis could in principle be applied to these peptides as well. Bradykinin, an unrelated peptide, also produces dips on luminal additions, but perhaps on a different receptor since no cross-tachyphylaxis was seen with substance P (Fig. 2). As with substance P, the relation between the dip and the basal potential difference was linear, suggesting that a similar process may be responsible (Fig. 2B).

As noted earlier, tight junctions may not be entirely passive elements, and a number of modulators of paracellular permeability have been reported [2–6]. Recently, using this same preparation, Al-Bazzaz and Yadava suggested that prostaglandins could decrease paracellular conductance across the tissue [6]. The effects reported here, however, are more rapid and transient and appear to involve the selective opening of an anion channel. The physiological significance has yet to be established, but these observations do add peptides to the list of possible modulators of junctional permeability in epithelia.

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