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Lipopolysaccharide from *Klebsiella pneumoniae* Inhibits Na^+ Absorption in Canine Tracheal Epithelium

J. TAMAOKI, N. SAKAI, K. ISONO, T. KANEMURA, K. TAKEYAMA, AND T. TAKIZAWA*

First Department of Medicine, Tokyo Women's Medical College, Tokyo 162, Japan

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The effect of lipopolysaccharide (LPS) from *Klebsiella pneumoniae* on the bioelectric properties of canine cultured tracheal epithelium was examined. LPS decreased short-circuit current (Isc), and its effects on Isc were reduced when Isc was inhibited by amiloride and indomethacin. We speculate that LPS may selectively inhibit Na^+ absorption through the inhibition of prostaglandin synthesis by airway epithelium.

Gram-negative bacteria and their cell envelope lipopolysaccharide (LPS) are present in a wide variety of occupational and general environments and have the capacity to interact with the cellular mediator systems of the host including platelets, mast cells, basophils, neutrophils, monocytes, macrophages, and endothelial cells. Through these interactions with the respiratory system, LPS has been proposed to contribute to the pathophysiology of pulmonary edema and airway inflammation (3, 9).

It is well known that hypersecretion of airway surface fluid is one of the characteristic features of bacterial infection and that water transport across the airway mucosa is regulated mainly by epithelial ion transport (6). Thus, to determine whether LPS from *Klebsiella pneumoniae* alters ion transport by airway epithelial cells, we studied the bioelectric properties of canine tracheal epithelium under short-circuited conditions in vitro.

After the submucosal tissue of the canine trachea was removed, the epithelial layer was digested with 0.1% protease type XIV (Sigma Chemical Co., St. Louis, Mo.) for 24 h, and epithelial cells were isolated by centrifugation (800 × *g*, 9 min) (12). The cells were then plated at a density of $1.5 \times 10^6/cm^2$ with 1 ml of Hamms nutrient F12 medium containing six hormones and 1% fetal calf serum per Linbro tissue culture plate (Flow Laboratories, Inc., McLean, Va.) and grown on Nuclepore polycarbonate filters at 37°C in a CO_2 incubator. On day 10 of incubation, cells were mounted between two Lucite half-chambers bathed with Krebs-Henseleit solution to measure electrical properties (1). The short-circuit current (Isc), a current sufficient to bring the spontaneous transepithelial potential difference to zero, was recorded continuously except for 3 s every 10 min when the voltage clamp was turned off and the potential difference was recorded. Tissue conductance (*G*) in millisiemens per square centimeter was calculated by dividing the measured Isc by the surface area (microamperes per square centimeter) and by the potential difference (millivolts). At the end of this experiment, aliquots (100 μl) from the bathing medium were analyzed for prostaglandin E_2 (PGE_2), a major product of arachidonic acid metabolism in canine tracheal epithelium (8), in duplicate by ^{125}I radioimmunoassay as described previously (10).

Addition of LPS from *K. pneumoniae* dissolved in saline to the submucosal solution (100 μg/ml) caused a rapid decrease in Isc within 1 to 2 min from 6.8 ± 0.9 to 4.3 ± 0.5 $\mu A/cm^2$ ($P < 0.01$, $n = 9$), accompanied by corresponding decreases in potential difference (2.0 ± 0.3 to 1.5 ± 0.2 mV, $P < 0.05$) and *G* (3.4 ± 0.4 to 2.8 ± 0.3 mS/cm^2, $P < 0.05$), whereas mucosal LPS had no effect. This inhibitory effect of LPS on Isc was dose dependent, the maximal decreases from the baseline value and 50% inhibitory concentration being 4.2 ± 0.6 $\mu A/cm^2$ ($P < 0.001$, $n = 7$) and 90 μg/ml, respectively (Fig. 1). Treatment of cells with furosemide (10^{-4} M) and diphenylamine-2-carboxylate (10^{-4} M) decreased Isc by 2.1 ± 0.3 and 2.4 ± 0.4 $\mu A/cm^2$, respectively ($n = 7$, in each case), and the subsequent addition of submucosal LPS (100 μg/ml) further decreased Isc (Fig. 2). On the other hand, amiloride (10^{-4} M) decreased Isc by 3.6 ± 0.7 $\mu A/cm^2$ ($n = 7$), and the decrease in Isc induced by LPS was less than that without amiloride pretreatment ($P < 0.001$). Pretreatment of cells with indomethacin (3×10^{-6} M) likewise reduced the inhibitory effect of LPS on Isc. The release of PGE_2 from tracheal epithelium was likewise inhibited by the submucosal addition of 100 μg of LPS per ml ($P < 0.05$, $n = 8$) but not by the addition of mucosal LPS (Fig. 3). Indomethacin was effective in inhibiting the release of PGE_2.

Tracheal epithelial cells have the capacity for both active Cl^- secretion and active Na^+ absorption, and the magnitude of these processes can be reflected by epithelial Isc (7). Although we did not directly measure ion fluxes, the results of the present study indicate that LPS from *K. pneumoniae* may selectively inhibit the absorption of Na^+ by canine tracheal epithelium. This conclusion is based on the findings that the decrease in Isc elicited by LPS was attenuated by pretreatment of cells with amiloride, a Na^+ channel blocker, but not by furosemide, an inhibitor of Cl^- transport, or diphenylamine-2-carboxylate, which inhibits the Cl^- conductance of the apical membrane (Fig. 2). In the presence of amiloride, the Isc appeared to be generated by Cl^- secretion. Because LPS was without effect on Isc in this condition, Cl^- movement may not be influenced by LPS. In the presence of furosemide or diphenylamine-2-carboxylate, LPS further decreased Isc, implying that the effect of LPS is Na^+ dependent.

The submucosal addition of LPS decreased Isc in a dose-dependent fashion, whereas it was without effect when added to mucosal side. The reason for this difference is uncertain, but one possible explanation would be that LPS receptors are localized on the submucosal but not mucosal membrane of canine tracheal epithelium. To confirm this, autoradiographic analysis of LPS-binding sites might be valuable.

Because Na^+ absorption is regulated by endogenous PGE_2 (4) and because LPS has been shown to inhibit epithelial PGE_2 production (5), we assessed the possible contribution of this prostanoid to the LPS action. We found

* Corresponding author.

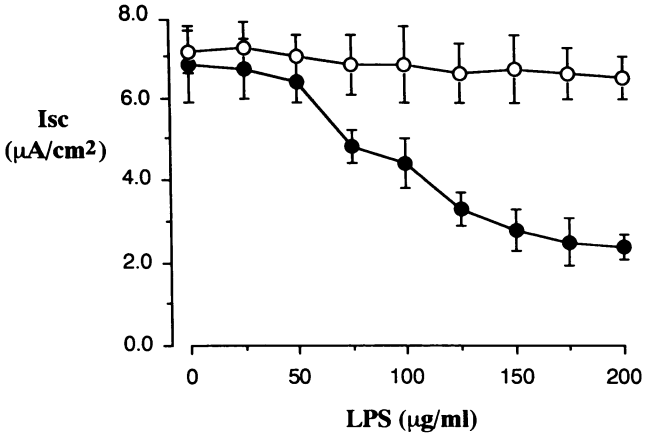

FIG. 1. Dose-dependent effect of LPS on Isc of canine tracheal epithelium. LPS was added to either the mucosal (○) or submucosal (●) solution. Each point represents mean ± standard error; $n = 7$.

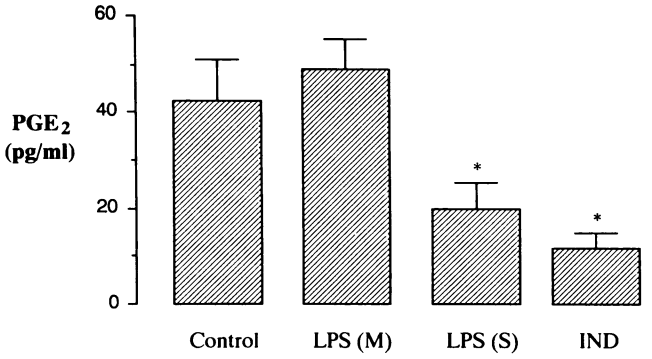

FIG. 3. Effects of LPS (100 μg/ml) and indomethacin (IND, 3×10^{-6} M) on PGE_2 release from canine tracheal epithelium. LPS was added to either the mucosal (M) or submucosal (S) side, and IND was added to both sides. After 30 min of drug addition, PGE_2 levels in the bathing medium was measured. *, $P < 0.05$, significantly different from control values (no drug added).

that indomethacin reduced the LPS-induced decrease in Isc (Fig. 2), suggesting that the effect of LPS is attributable to the inhibition rather than the stimulation of arachidonic acid conversion to its metabolites via the cyclooxygenase pathway, because PGE_2 has been shown to increase epithelial Isc (2). This notion is further supported by the finding that submucosal LPS decreased the release of PGE_2 from tracheal epithelial cells (Fig. 3).

It is known that Na^+ absorption and Cl^- secretion by the tracheal epithelium is correlated with the movement of fluid across airway mucosa (11). The lack of reactivity of epithelium to the mucosal LPS would benefit the host since mucosal surfaces are colonized with gram-negative organisms under normal circumstances. However, when bacterial infection causes airway epithelial damage such as the disruption of tight junctions, LPS could interact with the submucosal membrane of epithelium and the subsequent inhibition of Na^+ absorption might play a role in airway hypersecretion.

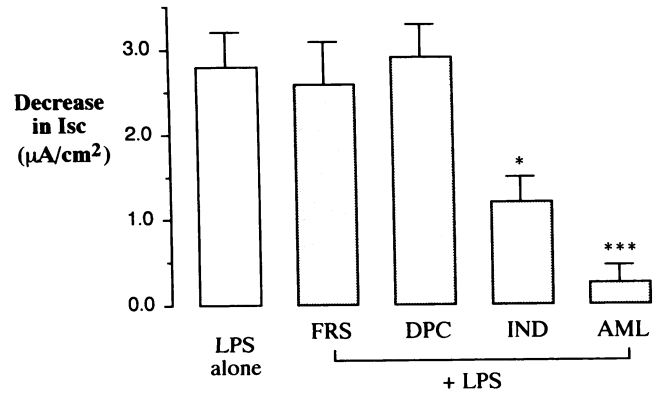

FIG. 2. Effects of furosemide (FRS, 10^{-4} M), diphenylamine-2-carboxylate (DPC, 10^{-4} M), indomethacin (IND, 3×10^{-6} M), and amiloride (AML, 10^{-4} M) on the LPS (100 μg/ml)-induced inhibition of Isc. Responses are expressed as the decrease in Isc from the values obtained before the addition of LPS. Values are means ± standard error; $n = 7$ for each group. *, $P < 0.05$; ***, $P < 0.001$, significantly different from the responses to LPS alone.

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