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Inhibition of amiloride-sensitive sodium conductance by indoleamines

(baboon bronchus/rat colon/short-circuit current/serotonin/melatonin)

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ABSTRACT **To examine a possible role of indoleamines in the regulation of epithelial sodium absorption, the effect of serotonin (5-hydroxytryptamine) and several derivatives on electrolyte transport was measured *in vitro* in the baboon bronchus and in the trachea and colon of sodium-deficient rats. Serotonin, melatonin (*N*-acetyl-5-hydroxytryptamine), and harmaline (1-methyl-7-methoxy-3,4-dihydro-β-carboline) inhibited sodium transport in all three preparations in a similar manner to the natriuretic agent amiloride. In all three epithelia, sodium absorption via the amiloride-sensitive pathway constitutes a substantial portion of total electrolyte transport, measured as the amiloride-sensitive short-circuit current. Thus, 25 μM amiloride inhibited the short-circuit current 21% in the rat trachea, 63% in the baboon bronchus, and 90% in the rat colon. Serotonin, melatonin, and harmaline inhibited the amiloride-sensitive portion of the short-circuit current from the luminal side of the epithelium. The inhibition was rapid, requiring only seconds, and maximal inhibition by serotonin was identical to that by amiloride. When sodium was omitted from the luminal solution, the short-circuit current was reduced a similar amount, suggesting that sodium absorption was being inhibited by both amiloride and the indoles. The IC_{50} value for amiloride was 50 nM in the baboon bronchus and 500 nM in the rat colon. In contrast, the IC_{50} value for serotonin was 0.4 mM in the baboon bronchus and 8 mM in the rat colon. These results, together with the wide distribution of amine-precursor-uptake-and-decarboxylation (APUD) cells in the respiratory and intestinal tract, suggest that certain indoleamines could play a role as local regulators of fluid and electrolyte transport. For example, in the airways, indoleamines may be one of the factors involved in regulation of the depth of the periciliary fluid layer.**

Amiloride is a potassium-sparing diuretic that, at micromolar concentrations, inhibits sodium absorption in many epithelia that scavenge sodium from the luminal or external side of the epithelium (1, 2). Amiloride inhibits sodium absorption by blocking a specific sodium channel or pore in the luminal plasma membrane of epithelial cells so that sodium cannot enter the cell from the luminal side (2–13). Because of its specificity, amiloride has been used to operationally define a certain type of sodium transport that can be quantitated as the amiloride-sensitive short-circuit current *in vitro* in epithelial preparations.

Epithelial sodium transport plays a crucial role in maintaining electrolyte balance in higher animals. The importance of this transport suggests that efficient regulatory mechanisms have evolved. However, no physiological compounds are known that can rapidly inhibit sodium absorption through the amiloride-sensitive pathway—i.e., one that acts similar to the drug. The currently known regulatory mechanism is through adrenal steroids that induce amiloride-sensitive sodium transport (1, 5). Regulation by adrenal steroids is on the time scale of hours (1, 14, 15), rather than seconds as in the case of amiloride. Because indoleamines show structural similarity to amiloride (see below) and because serotonin (5-hydroxytryptamine) and other indoleamines are present in relatively high concentrations in the mucosa of the respiratory and gastrointestinal tracts (16–19), we have investigated their effects on amiloride-sensitive sodium transport in the baboon bronchus, the rat trachea, and the rat colonic epithelium (20).

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METHODS

Animals and Tissues. First- to third-order bronchi were obtained within 15 min of death from adult male baboons (*Papio anibus*). Baboons had been obtained from various sources and had been subjected to right middle cerebral artery occlusions and various treatment protocols to alleviate the effect of the stroke. The treatments included in some cases prolonged (up to 4 days) pentobarbital anesthesia with respiratory support. Baboons were anesthetized with sodium pentobarbital at 30 mg/kg or ketamine hydrochloride at 10 mg/kg (or both) and were sacrificed by intravenous injection of saturated potassium chloride at 0.2 ml/kg.

Bronchi were placed in Hanks' balanced salt solution (21) buffered to pH 7.4 with sodium bicarbonate and gassed with 95% oxygen/5% carbon dioxide. Bronchial wall segments (≈5 × 10 mm) were stripped of their adventitia and mounted in a chamber similar to the one designed by Frizzell and Schultz (22). The tissue was maintained in bicarbonate-buffered Krebs–Henseleit balanced salt solution (23)/5 mM D-glucose at pH 7.4 and 37°C.

Male Sprague–Dawley rats (100–400 g) were obtained from Zivic–Miller Laboratories (Allison Park, PA). The animals were maintained on a sodium-deficient diet similar to one described by Hartroft and Eisenstein (1, 24). The animals were allowed to eat and drink deionized water ad lib. Rats were anesthetized with sodium pentobarbital (90 mg/kg) intraperitoneally. The descending colon or the trachea was removed, rinsed with oxygenated Krebs–Henseleit solution (23), and mounted in a Frizzell–Schultz chamber (22).

Electrical Measurements. Short-circuit current, transepithelial potentials, and tissue resistances were measured with an automatic voltage clamp (1, 25, 26) using calomel/saturated potassium chloride electrodes with 2% agar bridges as the potential electrodes and platinum wire electrodes as the current electrodes. The amiloride-sensitive portion of the short-circuit current was determined with an excess (≈25 μM) of amiloride on the luminal side of the tissue.

Ion Replacement Studies. A sodium-free Krebs–Henseleit solution was prepared by replacing sodium chloride and bicarbonate with iso-osmotic choline chloride and bicarbonate. After stable electrical values were obtained with the sodium Krebs–

Henseleit solution, the luminal or serosal baths were replaced with the sodium-free solution. Electrical values in the absence of sodium were determined after a 15-min equilibration period.

The dependence of the short-circuit current on the luminal sodium concentration was determined by gradually adding sodium Krebs–Henseleit solution to the sodium-free solution on the luminal side. Electrical values were determined as above and the sodium concentration was verified by flame spectrophotometry.

Treatment of Test Substances. Amiloride hydrochloride and indoles were dissolved in degassed Krebs–Henseleit solution and adjusted to pH 7.4. Indoles were prepared fresh within 30 min of use. All additions were made to the luminal side of the tissue.

Materials. Amiloride hydrochloride was the gift of Merck, Sharp & Dohme. Scillaren was donated by Sandoz Pharmaceutical. Indoles were purchased from Sigma. Sodium pentobarbital (Diabutal) was obtained from Diamond Laboratories (Des Moines, IA). The sodium-deficient rat diet (catalogue no. 902902) was prepared by the Life Sciences Group of ICN. All other chemicals were of reagent grade or better and were bought from various suppliers.

RESULTS

The electrical properties of baboon bronchus and rat trachea are reported here, while electrolyte transport by the rat colon has been extensively described (1, 14, 27). Table 1 summarizes the electrical properties of the epithelia used in this study. All three tissues spontaneously generated a transepithelial potential (contraluminal side positive) and a short-circuit current. The average short-circuit current was 43 $\mu A/cm^2$ in the baboon bronchus, 19 $\mu A/cm^2$ in the rat trachea, and 422 $\mu A/cm^2$ in the rat colon. The short-circuit current of trachea and colonic epithelium has previously been reported to be sensitive to cardiac glycosides added to the contraluminal side of the tissue (1, 28, 29). For the baboon bronchus, this property was specifically investigated in three experiments. Addition of 1 mM scillaren (a cardiac glycoside) to the contraluminal side inhibited the transepithelial potential and the short-circuit current by 95 ± 5%. Evidently, the ion movements responsible for the transepithelial potential and the short-circuit current are driven by the Na^+/K^+ ATPase located in the basolateral plasma membrane of the bronchial epithelial cells (30). The resistance values were intermediate between those typical for very "leaky" and very "tight" epithelia: 63 $\Omega \cdot cm^2$ for the rat colon, 88 $\Omega \cdot cm^2$ for the baboon bronchus, and 142 $\Omega \cdot cm^2$ for the rat trachea.

The observed short-circuit current and the transepithelial potential could be due to an excess of luminal-to-serosal cation movements or to serosal-to-luminal anion movements. To determine which of these situations is occurring in the baboon bronchus and the rat trachea, we have used amiloride as an inhibitor of net luminal-to-serosal sodium transport (1–4). After addition of amiloride to the luminal side of the tissue, the transepithelial potential and the short-circuit current are rapidly reduced (Fig. 1). The normal baboon bronchus apparently possesses an amiloride-sensitive sodium transport pathway. In contrast, the trachea and colon of rats on a laboratory diet containing the normal amount of sodium have little of this type of sodium transport (data not shown for trachea; for rat colon, see refs, 1, 14). However, amiloride-sensitive sodium transport can be induced by secondary hyperaldosteronism (1, 5, 14, 31). Therefore, tissues from sodium-deficient rats were used in this study. The observed amiloride sensitivity of the tissues in this study were 21% for rat trachea, 63% for baboon bronchus, and 90% for rat colon. The rapid inhibition observed with amiloride is consistent with an effect on the luminal membrane; no inhibition was observed when excess amiloride (up to 1 mM) was added to the serosal side of any of the tissues (results not shown) (7). The mechanism of amiloride action as a specific inhibitor of sodium current has been well established in various species (1–13, 27). The effect of amiloride in the present studies was similar to that in studies in which the specificity for sodium transport has been rigorously established (3, 4, 6–8). The similarity was present in terms of inhibition from the luminal side only and of a 50% effective dose of <1 μM. Therefore, it is reasonable to conclude that the amiloride-sensitive short-circuit current represents sodium absorption.

To directly determine whether the short-circuit current was sodium dependent, the concentration of sodium was varied on the luminal side of the tissue. The results with baboon bronchus are shown in Fig. 2. The short-circuit current in the virtual absence of sodium ($\approx$16 $\mu A/cm^2$) was similar to that in the presence of sodium and supramaximal concentrations of amiloride. Amiloride did not affect the short-circuit current in the absence of sodium. The two observations together indicate that sodium is required for the amiloride-sensitive portion of the current. The residual part may be due to net, serosal-to-luminal, anion movements (25). Similar results have been obtained with the rat colon (data not shown).

Addition of serotonin to the luminal side of the baboon bronchus (Fig. 1) or to the trachea or colon from sodium-deficient rats (results not shown) reduced the transepithelial potential and the short-circuit current in a rapid and reversible manner similar to that of amiloride. The inhibition was concentration dependent (Fig. 3) and was limited to the amiloride-sensitive

Table 1. Electrical properties of selected epithelia

Property	Tissue: Baboon bronchus (n = 12)	Rat trachea (n = 3)	Rat colon (n = 12)
Short-circuit current, $\mu A/cm^2$	43 ± 7	19 ± 11	422 ± 227
Transepithelial potential, mV	3.9 ± 1.5	2.4 ± 0.7	22.1 ± 3.6
Resistance, $\Omega \cdot cm^2$	88 ± 35	142 ± 88	63 ± 25
Amiloride-sensitive short-circuit current, % total	63 ± 17	21 ± 14	90 ± 4

Results are mean ± SD.

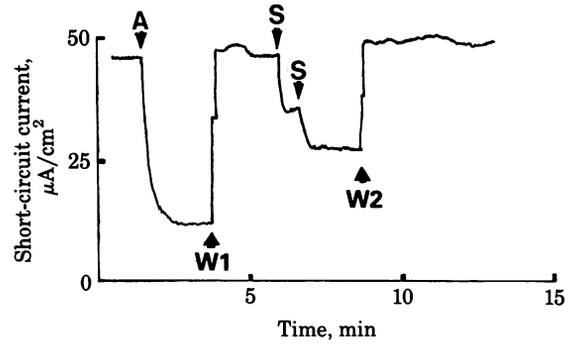

FIG. 1. Inhibition of short-circuit current of baboon bronchial epithelium by amiloride and serotonin. Short-circuit current tracing is from a typical tissue. Amiloride (A) at 1 μM was added to the luminal bath; at W1 (wash), the bath was changed to a drug-free solution. Serotonin (S) was added to final concentrations of 0.3 and 0.6 mM and was removed at W2.

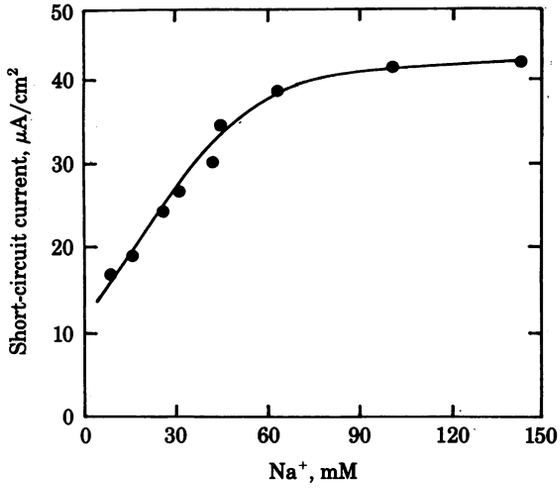

FIG. 2. Sodium dependence of short-circuit current of baboon bronchial epithelium. Luminal sodium in Krebs–Henseleit solution was replaced by choline. Similar results were obtained if the sodium concentration was increased or decreased during the course of the electrical measurements.

portion of the short-circuit current (Fig. 1). In other words, at submaximal doses of one inhibitor, addition of the second further decreases the short-circuit current. However, in the presence of excess amiloride (≈25 μM), subsequent addition of serotonin had no further inhibitory effect. The concentration dependence of the inhibition by serotonin and melatonin parallels that by amiloride (Fig. 3) (rat trachea and rat colon data not shown), consistent with a similar mechanism of action. Interestingly, the short-circuit current of the colon from rats on a diet containing a normal amount of sodium was not inhibited by serotonin added to the luminal solution. This tissue has little or no amiloride-sensitive sodium transport (1, 14).

The inhibitory effect was not specific for serotonin but was also observed with other indoleamines, such as melatonin (*N*-acetyl-5-methoxytryptamine), tryptamine, and harmaline (1-methyl-7-methoxy-3,4-dihydro-β-carboline). Relative efficacies of the different indoleamines in the baboon bronchus and rat colon preparations are shown in Table 2. The respiratory

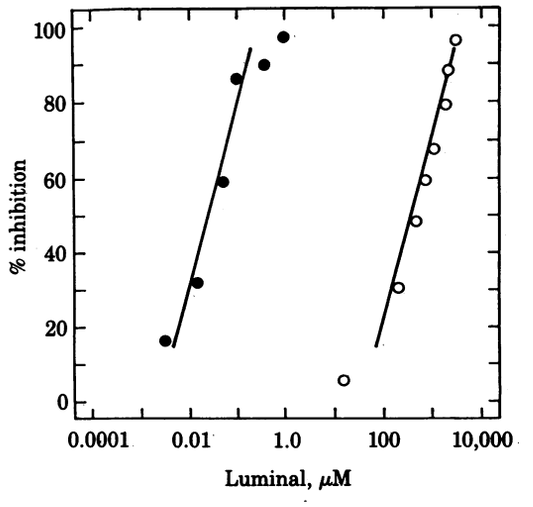

FIG. 3. Relative efficacy of amiloride and serotonin at inhibiting amiloride-sensitive short-circuit current of baboon bronchial epithelium. Drugs were added to the luminal side of the epithelium. The short-circuit current inhibitable by amiloride was 41 μA/cm².

Table 2. Efficacy of indoleamines and related compounds to inhibit amiloride-sensitive short-circuit current

Compound	IC_{50}, μM	
	Baboon bronchus	Rat colon
Amiloride	0.05	0.5
Harmaline	400	3,000
Melatonin	700	6,000
Serotonin	400	8,000
Tryptamine	8,000	25,000

Results are averages for 3–10 animals; the variability among animals was ≈3-fold.

epithelia seem to be more sensitive to both amiloride and the indoleamines by approximately an order of magnitude. Serotonin was the most effective indoleamine in the baboon bronchus with an IC_{50} value of 0.4 mM, while harmaline had the greatest potency in the rat colon with 3 mM necessary for half-maximal inhibition of the short-circuit current.

DISCUSSION

The observation of naturally occurring inhibitors of sodium absorption similar to amiloride is of substantial interest for the physiology of exocrine epithelia. The presented results indicate that luminal indoleamines inhibit epithelial sodium absorption in a manner similar to amiloride. The major observations supporting this conclusion are that (*i*) luminal indoleamines rapidly and reversibly inhibit the amiloride-sensitive short circuit current; (*ii*) supramaximal doses of amiloride and indoleamines together do not produce a synergistic effect; (*iii*) inhibition by both amiloride and indoleamines depends on the presence of luminal sodium; and (*iv*) the inhibitory effect of indoleamines is present only in tissues that have amiloride-sensitive ion transport; in epithelia that normally do not have this type of transport (normal rat trachea or colon), induction of amiloride-sensitive ion transport by secondary hyperaldosteronism (sodium deficiency) is associated with that of indoleamine-inhibitable ion transport. Furthermore, both amiloride and the effective indoleamines have an unsaturated heterocyclic ring system and two nitrogens in approximately the same positions (Fig. 4). This structural similarity suggests that they may affect the same inhibition site.

The similarity of action between amiloride and certain indoleamines suggests that some indoleamines may play a role as a naturally occurring regulator of the amiloride-sensitive sodium transporter. Serotonin has been measured in human bronchial fluid at 2–8 times the concentration in the serum, and serotonin has also been found to be secreted into the lumen of the gastrointestinal tract (32, 33). However, a definite role of indoleamines cannot be evaluated at this time because of the difficulty in determining luminal concentrations. Nevertheless, the luminal secretion of indoleamines could locally reach sufficient levels for effective inhibition of sodium absorption, particularly in the respiratory epithelium, in which the epithelial cell surface is large in comparison with the volume of the luminal fluid layer. The physiology of electrolyte and water transport in the respiratory tract is also consistent with the presence of a regulator of ion transport that has properties similar to serotonin. This will be therefore discussed in more detail.

The results from baboon bronchus and trachea of sodium-deficient rats (Figs. 1 and 3 and Table 2) indicate that the upper respiratory tract has a substantial capacity to actively reabsorb sodium via an amiloride-sensitive pathway (see also refs. 34–36), presumably by a specific sodium transporter in the luminal plasma membrane of the epithelial cells. The presence of sodium reabsorption in the bronchi was predicted by studies

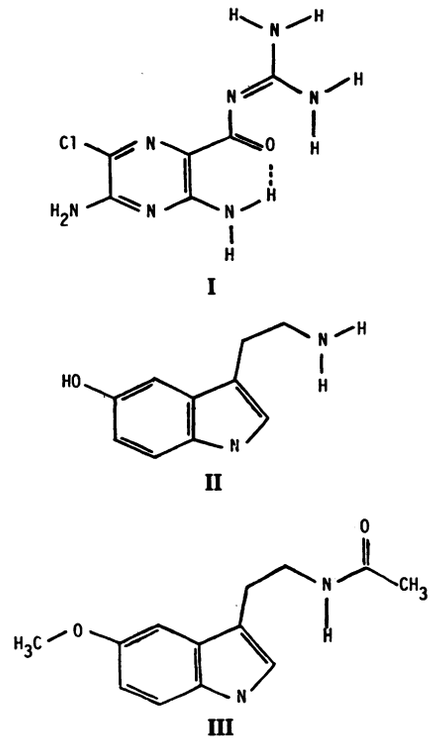

FIG. 4. Structures of amiloride (I), serotonin (II), and melatonin (III). Structures are drawn to emphasize similarities.

of the flow of periciliary fluid from the small broncheoli to the primary bronchi (37, 38). As movement from the small toward the large bronchi and the trachea occurs, the total circumference of the airways decreases and electrolyte reabsorption is required to regulate the amount of respiratory fluid. Conversion of the amiloride-sensitive short-circuit current in baboon bronchus to fluid absorption under *in vivo* conditions yields 1.5–2 $ml \cdot min^{-1} \cdot m^{-2}$ of epithelial surface (35).

The established regulatory mechanism of amiloride-sensitive sodium transport in a variety of epithelia is through mineralocorticoids (1, 14, 27). This regulation also seems to hold for the respiratory tract as sodium deficiency, which is associated with secondary hyperaldosteronism, was required for the demonstration of amiloride-sensitive ion transport in the rat trachea. In this respect, the trachea is similar to the intestinal tract (1). This regulation of epithelial salt transport by mineralocorticoids exposes the respiratory tract to two potential problems: (*i*) modulation of mineralocorticoid levels occurs in response to overall body needs for sodium and (*ii*) regulation is slow with a response time of hours to days (1). However, because the respiratory tract is exposed to conditions that require rapid changes in hydration (e.g., dry air panting), there is substantial need for a local and rapid mechanism to regulate salt and water transport. One important physiological situation requiring rapid regulation of fluid transport in the airways is birth. Interestingly, recent studies in fetal lambs show that the bronchial amiloride-sensitive pathway is rapidly activated in fetal bronchi during labor or by the infusion of epinephrine (39).

The inhibition of sodium absorption by indoleamines suggests that serotonin or a derivative may regulate the depth of the periciliary fluid layer of the respiratory epithelium *in vivo*. If this layer becomes too shallow as a result of dehydration, the inhibition of sodium absorption would, by unmasking any secretory process, increase its depth.

The indoleamines are well suited as local regulators because they can be synthesized, released, and metabolized locally (16, 18). A variety of enterchromaffin cells or amine-precursor-and-decarboxylation (APUD) cells are located in the epithelium of both the respiratory and gastrointestinal tracts (40–44). In the bronchi, the enterochromaffin cell population peaks in fetal life (42, 43). Characteristic fluorescent and other histochemical reactions have identified these as serotonin-containing cell populations in both epithelia (40, 43). In the rat colon, melatonin has been demonstrated by immunohistochemical methods (44). Clues to the physiological functions of indoleamine-containing cells may be obtained by comparison of the occurrence of these cells or pathological serotonin secretions. Thus, the higher density of enterochromaffin cells in fetal life (42, 43) may be related to the higher fluid production of the fetal respiratory tract in comparison with the adult and the presence of mechanisms to rapidly decrease this fluid output with birth.

On the other hand, it is well established that serotonin-secreting tumors are associated with an altered secretory and absorptive balance resulting in diarrhea and bronchorhea (45, 46). This association of serotonin with increased epithelial salt and fluid secretion has generally been explained on the basis of serotonin as a secretagogue (16, 47). Our studies suggest that serotonin has a second, complementary, activity—namely, inhibition of sodium transport—that could also contribute to increased salt and fluid secretion by epithelial organs.

The difference between the inhibitory effect of serotonin on sodium reabsorption and the stimulatory effect on salt (i.e., chloride) secretion can be well demonstrated in the rat colon. The short-circuit current from the colon of control rats with sufficient dietary sodium, which is not inhibitable by amiloride and serotonin (ref. 1 and this report), is stimulated by serotonin (48). This stimulatory effect is known for several types of epithelia (16, 45, 47) and is apparently brought about by interaction of serotonin with a receptor on the contraluminal plasma membrane and intracellular second messengers such as calcium in the small intestine (49). Interestingly, the secretory response to serotonin—i.e., stimulation of the short-circuit current—is no longer present in the colon of sodium-deficient rats, suggesting that the cellular response depends on the physiological state or type of epithelial cells (48). In both cases, serotonin effects an increase in the secretory-to-absorptive balance. From the point of view of energy conservation, it is advantageous to first block any active electrolyte absorption in the tissue before initiating actual secretion.

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