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Colchicine Effect on the Permeability of the Whole Epithelium and of Isolated Cells of Frog Skin

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Abstract

The effect of 2×10^{-5} M colchicine on epithelial cells isolated from frog skin was investigated. Three hours of treatment with colchicine did not change either Na^+ and K^+ content of isolated cells or nonelectrolyte permeability. When ADH (50 mU/ml) was added, thiourea uptake values became greater than without the hormone; the same values were found in the cells previously treated with colchicine. Na^+ transepithelial transport, measured by means of short-circuit current, was inhibited by the antimitotic agent both under control conditions and after ADH stimulation. These results support the view that colchicine does not directly affect ADH action on membrane permeability, but influences some mechanism that controls ADH action on transepithelial transport. Intercellular junctions appear to be the location of such a mechanism.

Introduction

Vasopressin promotes transepithelial water and sodium transport through the mammalian renal tubule (1), amphibian skin (2) and amphibian bladder (3–8). Such an effect seems to be cAMP-mediated (8–9), but the permeability enhancement mechanism is still unclear.

Recently, the cellular mechanism by means of which cyclic AMP regulates water permeability has been extensively studied. Taylor et al. (18) have reported that colchicine, (11) an antimitotic agent which exerts disrup-

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tive effects on microtubules (MT), also strongly inhibits the action of vasopressin on osmotic water movement through toad bladder, without affecting Na^+ active transport. In view of this, the role of microtubules in ADH action has been investigated.

The point which still remains unclear is: is the microtubular system responsible for ADH action on outer membrane permeability, or is it involved in some other mechanism controlling ADH action on permeability?

In a previous paper (19), the effect of ADH on nonelectrolyte permeability through frog skin was reported. Our results indicate that: (1) ADH treatment results in a symmetrical increase of both nonelectrolyte fluxes; (2) such an effect is mimicked by db-cAMP; (3) colchicine treatment strongly inhibits the effect of ADH and cAMP. These results suggest that the involvement of microtubules presumably occurs subsequent to the formation of cyclic AMP.

It may be possible that microtubules are involved in the control of permeability through such a mechanism: (1) they may not be influenced by cAMP, but instead serve as a cytoskeleton which directs the translocation of other components which regulate permeability (10); (2) their structure and function may be influenced by vasopressin-dependent cAMP and they may affect permeability within the membrane more directly by interacting with other components; (3) they may play a role in cell-to-cell exchange. (7, 17).

In order to investigate this problem, we have studied the effect of colchicine on the response to ADH of epithelial cells isolated from frog skin. The effect of colchicine on transepithelial Na^+ transport is also reported.

Materials and Methods

Experiments were performed on frogs (*Rana esculenta*) that had been kept in running tap water at room temperature prior to the experiments.

Cell Isolation

Cells were isolated with a small modification of the Zylber et al. method (20).

Briefly: The tissues from 10–15 frogs were dissected and a series of incisions of the corion were made with a stainless steel blade. The skins were then placed on the bottom of a smooth-edged tubular chamber (21). The outer side of the skin was bathed with a solution containing 85 mM NaCl; 4 mM KCl; 17.5 mM $NaHCO_3$; 0.8 mM KH_2PO_4; 10 mM glucose; 0.01 g% penicyllin, 0.1 g% streptomycin (Solution 1), pH = 7.6. The inner side was bathed with Solution 2 (Solution 1 + 0.2% trypsin).

After 30-min incubation at room temperature, with the aid of a smooth-edged microscope glass, large pieces of epithelium were removed. The epithelium was put into Solution 3 (Solution 1 + 0.03 g% trypsin) for 30 min at 37°C and was stirred continuously. The supernatant was filtered through four pieces of gauze and spun for 8 min at 800 × *g*. The sediment was washed twice with Solution 1 and finally resuspended in Solution 4 (Solution 1 + 1 mM $CaCl_2$ + 5 mM $MgCl_2$ + 0.1 g% lima-bean trypsin-inhibitor).

The isolated cells were diluted and counted in a Thoma chamber for blood cell counting. Cells were examined under a microscope to estimate both the number of deformed and destroyed cells as well as the amount of released subcellular particles. The percentage of cells that had not absorbed Trypan blue (0.2% in Solution 1) was also determined. This percentage was 90–93%.

Uptake Experiments

Isolated cells, after 30-min equilibration in Solution 4, were used for uptake experiments.

Four hundred μl of a medium containing Solution 4 + 1.5 mM thiourea + 3 μCi/ml of ^{14}C-thiourea were added to 200 μl of the cell suspension (0.8–1.0 mg protein). Throughout the procedure the incubation media were oxygenated and stirred at 37°C.

At different periods an aliquot (50 μl) of the cell suspension was removed and squirted into a glass tube containing 2.5 ml of ice-cold Solution 1 and immediately filtered through a Millipore apparatus. Filters were dissolved in 10 ml of a scintillation solution (1000 ml toluene, 500 g Triton-X-100, 75 ml CH_3COOH, 8 g PPO), and the radioactivity was measured in a Tri-Carb Packard scintillation spectrometer.

An aliquot of cell suspension was homogenized with a Potter-Elvehjem homogenizer for 2 min. Triton-X-100 (final concentration, 0.1%) was added to the homogenate and then kept at 37°C for 2 hr. Aliquots of the homogenate were analyzed for cell protein by the Lowry method (16), using bovine serum albumin as a standard.

In ADH experiments, the hormone (final concentration, 50 mU/ml) was added to the preincubation medium 30 min before the beginning of "uptake." For colchicine experiments 2×10^{-5} M colchicine was added 3 hr before commencement.

Short-Circuit Current Experiments

Short-circuit current (SCC) measurements were made by mounting the frog skin between two lucite chambers containing 7 ml of incubation medium and gassing with air at 22 ± 2°C, according to the Ussing and Zerahn technique (22).

In the experiments with colchicine, 2×10^{-5} M colchicine was added to the inner bathing fluid 3 hr before measurements were taken. Half of the ventral skin was used, the other half serving as a control.

In ADH experiments 50 mU/ml of the hormone was added to the inner side solution.

Na^+ and K^+ Measurements

Sodium and potassium content were measured with an EEL flame photometer. 1–2^{3}H Polyethylenglycol 4000 was used as extracellular marker.

Results

Figure 1A shows the effect of 2×10^{-5} M colchicine, added 3 hr before the experiments were begun, on thiourea uptake into isolated epithelial cells. If uptake values with and without colchicine treatment are compared, it can be seen that colchicine does not impair the permeability of isolated cells.

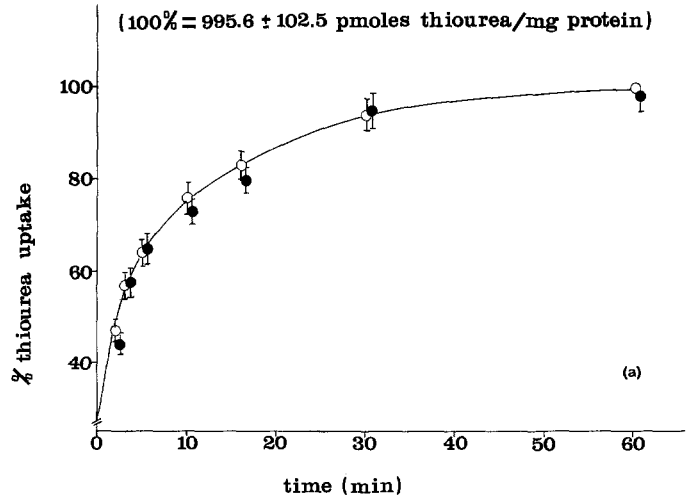

Fig. 1. Thiourea (1 mM) uptake by frog skin isolated epithelial cells for a period of 1 to 60 min. To standardize the experiments, the values are expressed as percentages of the control value obtained after 60-min incubation (100%). Curves are hand-drawn for the best fit of experimental readings. (a) ○ Control; ● in the presence of colchicine (2×10^{-5} M, 3-hr preincubation). (b) ○ Control; △ in the presence of ADH (50 mU/ml, 30-min preincubation). (c) △ In the presence of ADH (50 mU/ml, 30-min preincubation); ▲ in the presence of colchicine and ADH (2×10^{-5} M colchicine, 3-hr preincubation, and 50 mU/ml ADH, 30-min preincubation). Each point represents the mean ± SEM of eight to nine experiments.

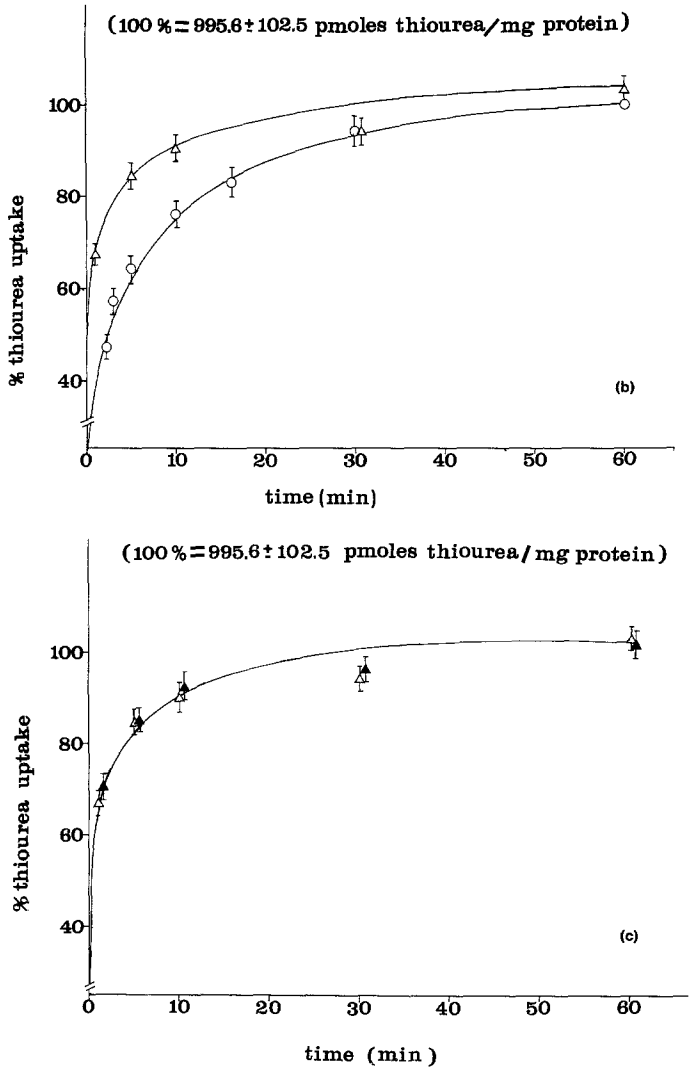

Fig. 1. Continued.

Since colchicine interacts with the microtubular system, such a finding suggests that microtubules are not involved in nonelectrolyte permeation in isolated epithelial cells. On the other hand, the failure of colchicine to alter thiourea uptake rules out the possibility that colchicine may affect cell intactness.

In order to analyze functional intactness, before and after colchicine treatment, water, sodium, and potassium content was measured. As shown in

Table I. Na^+ and K^+ Concentrations (in mEq/liter) of Isolated Cells Under Various Experimental Conditions

Experimental conditions	Number of experiments, *n*	Sodium[d]	Potassium[d]
Control	5	30.6 ± 1.9	106.6 ± 5.9
2×10^{-5} M colchicine preincubation[a]	5	31.9 ± 1.5	106.2 ± 1.7
50 mU/ml ADH[b]	3	32.2 ± 1.0	105.6 ± 2.0
50 mU/ml ADH after 2×10^{-5} M colchicine preincubation[c]	3	30.9 ± 1.7	103.9 ± 1.9

[a]Isolated cells were preincubated with 2×10^{-5} M colchicine for 3 hr before measurement was begun.
[b]ADH (50 mU/ml) was added to isolated cells 30 min before measurement.
[c]ADH (50 mU/ml) was added 30 min before measurement to isolated cells treated with 2×10^{-5} M colchicine for 3 hr.
[d]Means ± SE are reported.

Table I, isolated epithelial cells exhibit acceptable levels of K^+ and Na^+ concentrations. On the other hand, these levels, which are indices of cell membrane integrity, are not affected by colchicine.

Figure 1B shows ADH effect on thiourea uptake. It can be noted that ADH, added 30 min before the experiments were begun, enhances epithelial cell uptake as compared with that measured in control conditions.

ADH does not increase cell water content (M. Svelto, unpublished

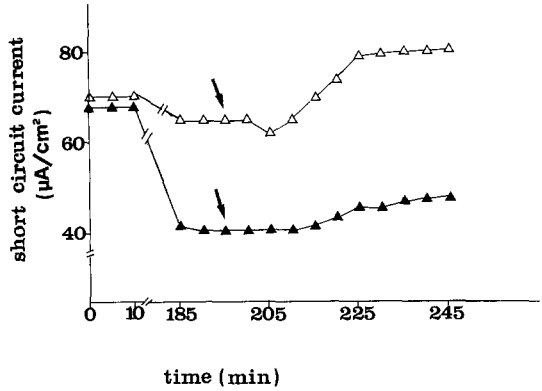

Fig. 2. Effect of ADH (50 mU/ml in the inner side solution) on short-circuit current through frog skin. △ ADH added in control conditions; ▲ ADH added to skin preincubated for 3 hr with 2×10^{-5} M colchicine. ADH addition is indicated by the arrows.

observations) reported in amphibian bladder (12, 14). Thus, increased uptake of thiourea per mg protein reflects a true permeability enhancement.

The colchicine effect on the response to 50 mU/ml of ADH is shown in Fig. 1C. It is evident that uptake values after ADH stimulation are virtually the same whether colchicine pretreatment has been carried out or not.

Figure 2 shows the effect of colchicine on Na^+ transepithelial transport measured by means of short-circuit current, with and without ADH stimulation. It can be seen that colchicine lowers basal and ADH-stimulated Na^+ transport.

Discussion

In a previous work carried out on the whole epithelium of frog skin, our results indicated that ADH enhances nonelectrolyte permeability and that such an effect is completely nullified by colchicine pretreatment (19). Similar results were obtained when db-cAMP was used. However, colchicine alone does not change nonelectrolyte permeability of frog skin (19, 23). Since colchicine exerts disruptive effects on microtubules, it seems most likely that the microtubular system plays a key role in ADH action. Such a role may be carried out subsequent to cAMP production. Microtubular-induced exocytosis of a surface-active agent that might increase mucosal membrane permeability has been suggested (4, 24, 25). Therefore, a possible explanation for our results is that colchicine inhibits the exocytotic transport of a surface-active agent responsible for ADH action on permeability. Experiments with the washing-out technique (19), however, showed that addition of ADH results in an abrupt increase of thiourea discharge and this effect is also present in colchicine-treated skin. The results of these experiments support the view that colchicine does not directly affect ADH action on outer membrane permeability, but influences some mechanism which regulates ADH action on transepithelial permeability.

Apart from the anatomical complexity of frog skin, it is difficult to interpret the results obtained when dealing with the whole epithelium. However, this problem has been overcome due to the possibility of isolating cells from the skin. Epithelial cells, isolated as previously reported, provide a simpler medium for the study of hormonal control of membrane permeability.

In order to state whether the inhibition of ADH effect on permeability of the whole epithelium is a primary effect of colchicine or results from ionic or other disturbances possibly linked with alterations in plasma membranes, the experiments described in Fig. 1A and Table I were carried out.

Sodium and potassium content was unaffected by colchicine and uptake

values were the same both with and without colchicine. On the other hand, these results, together with the fact that colchicine does not impair nonelectrolyte transepithelial permeability (in the absence of ADH), provide additional evidence that the colchicine effect is a primary one.

ADH enhances thiourea permeability of isolated epithelial cells and colchicine preincubation for a length of time sufficient to disrupt microtubules does not affect the response to the hormone. The absence of colchicine effect on thiourea permeability in isolated epithelial cells would favor the view that microtubules are not associated with ADH regulation of membrane permeability of isolated cells. Therefore, colchicine inhibition of ADH action on nonelectrolyte transepithelial permeability (19) may influence some function other than membrane permeability.

It has been suggested that junctional complexes between epithelial cells provide intracellular exchange of electrolytes (13, 15) which results in more or less coupled behavior and functional similarity of neighboring cells. In epithelia, such as frog skin, that consist of several layers, electrolyte exchange through low-resistance junctions between the different cell layers would, in addition, allow participation of all layers in transepithelial transport.

Na^+ exchange between different layers of frog skin epithelium has recently been demonstrated (26). Microelectrode experiments (6) show that low-resistance connections between different cell layers exist even under normal conditions.

Mills et al. (5) proposed a model for Na^+ transport through frog skin involving intercellular communication between different cell layers. Such communication should restrict ADH action on nonelectrolyte transepithelial permeability.

Recently, we proposed that colchicine can impair intercellular communication (19). According to this hypothesis, the limiting factor in nonelectrolyte movement through the skin is the rate of permeation across the outer membrane. The ADH-induced enhancement of outer membrane permeability should therefore render cell-to-cell rate of transport the limiting factor and this could be the site of colchicine action.

However, the present hypothesis is almost completely based on the different effects of colchicine on nonelectrolyte permeability of whole tissue and isolated epithelial cells.

The interpretation of the former effect could be complicated by the presence of two parallel permeation pathways for thiourea: cellular and paracellular pathways. At present, it is not easy to evaluate the quantitative role of these paths.

Our hypothesis, therefore, was tested on a true cellular phenomenon, i.e., Na active transport. SCC measurements show that transepithelial Na active

transport in the presence of colchicine is lowered both with and without ADH. However, in the isolated epithelial cells used, there was no evidence of sodium pump inhibition as cellular concentrations of Na^+ and K^+ were not modified by colchicine treatment either with or without ADH. Thus, the effect of colchicine on whole tissue and on isolated epithelial cells, concerning Na^+ active transport, is different. This effect is also consistent with the above hypothesis, but, to be proved, a more direct approach may be necessary. It remains of interest that colchicine does not affect urea and Na^+ permeability across toad urinary bladder (27, 28) which is an epithelium lined by a single cell layer.

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