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NOCODAZOLE INHIBITION OF THE VASOPRESSIN-INDUCED WATER PERMEABILITY INCREASE IN TOAD URINARY BLADDER

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Nocodazole is a synthetic antitumor drug that binds rapidly to tubulin. When this drug is applied to toad bladder prior to vasopressin stimulation it inhibits the vasopressin response. A maximum inhibition (68%) is reached with a dose level of 10 μg/ml applied one-half hour prior to vasopressin stimulation (20 mU/ml). This compares with an inhibition of 50% seen with a 3-h exposure of the tissue to colchicine (0.1 mM) prior to stimulation with vasopressin. Application of nocodazole (1 μg/ml) 3 min after hormonal stimulation shows no inhibition of the response at one-half hour past stimulation, These data support the view that microtubules are involved in the vasopressin-induced increase in water permeability in toad bladder and also indicate that this involvement is limited to the period prior to or directly after stimulation.

Introduction

Vasopressin (antidiuretic hormone) is known to increase the hydraulic conductivity of sensitive epithelia. This response is commonly measured as an increase in permeability to water of the urinary bladder of the toad, *Bufo marinus* [1]. Although the complete nature and sequence of intracellular events that bring about this change in permeability is still unknown, a strong case has been made for the involvement of microtubules in this process [2–5]. This argument is built upon observations from experiments using microtubule disrupting drugs to inhibit the increase in permeability elicited by vasopressin. The ability of these agents to inhibit the increase in permeability was shown to correlate with the destruction of microtubular structure in the cell. However, in assigning a role for the cytoskeletal elements from such data a note of caution is always sounded in that the inhibition of the permeability response by antimitotic drugs could be due to their interaction with other cellular structures and not from the disruptive effect on microtubules.

The work of Beebe et al. [6] on the in vitro elongation of chick lens epithelial cells has borne out these cautionary statements. The early phase of this elongation can be blocked by colchicine [7], vinblastine [6], demecolcine and podophyllotoxin [6]. The inhibition of elongation took place along with the disruption of the microtubular array and so microtubules were assigned a role in the elongation of these cells [7]. However, Beebe et al. [6] report that nocodazole, a synthetic microtubule disruptive drug, dismantles the microtubular apparatus in the embryonic chick lens epithelial cells without preventing their elongation. Further, it was demonstrated that colchicine in levels below that needed to disrupt microtubules still prevented the elongation of the chick cells [6]. It was shown that treatment of these cells with lumicolchicine did not prevent cell elongation. The effect of colchicine that blocks the process of cell elongation and its associated volume increase can then be seen to be specific for colchicine but independent of its ability to disrupt cytoplasmic microtubules.

In the toad bladder the vasopressin-dependent

increase in the permeability of the luminal membrane has also been thought to involve microtubules [4]. In light of this it would seem prudent to investigate the effect of nocodazole on the vasopressin-induced increase in transtissue water movement in toad bladder. The results of such studies are reported here and it has been found that exposure of bladder tissue to nocodazole in a manner that would disrupt microtubules also causes an inhibition of the hydro-osmotic response of toad bladder to vasopressin.

Materials and Methods

Experimental tissue was obtained from the Columbian toad, *Bufo marinus*, that had been acquired through commercial sources. The animals were kept in a tank on a bed of wet peat moss with free access to water until used. They were sacrificed by double pithing and the urinary bladder removed.

Osmotic water flow was assessed by gravimetric techniques. Measurements were obtained using the bag technique of Bentley [1] or through the use of a sealed chamber. The chamber consisted of a plastic tube 2.5 cm in length and 3 cm in diameter. One end of the tube was sealed with a thin, flaccid piece of rubber held with an 'O' ring and glued in place with epoxy. The chamber was filled with toad Ringer's solution (Na, 111.2; Cl, 113; K, 5.4; Ca, 1.78; HPO_4^{2-}, 4.8, $H_2PO_4^-$, 0.6 mequiv./l; pH 7.3; 220 mosM) and bladder tissue was stretched across the other end of the tube. The bladder was sealed in place with an 'O' ring. The chamber, with mucosal side exposed, was then immersed in 1/10 Ringer's solution. A port (rubber serum stopper) was placed in the side of the chamber to allow for the injection of hormone or drugs. The hemibladder bag, serosal side exposed, was placed in aerated full strength Ringer's solution and filled with a 0.1 strength Ringer's solution. The osmotic water gain was measured by removing the bag or chamber at specific intervals, blotting in a uniform manner and weighing (±10 mg). These data obtained from the bag or chamber techniques were found to be equivalent and so were pooled for analysis. An appropriate amount of DMSO was added to the control chamber in all experiments using nocodazole. When colchicine (Sigma) was used as the experimental drug, a 3-h incubation period was allowed before injection of the hormone. In all cases the tissue was stimulated by vasopressin at a final concentration of 20 mU/ml. Results reported as mean ± S.E. of the mean (number of preparations).

TABLE I

INHIBITION OF VASOPRESSIN-INDUCED WATER FLOW BY NOCODAZOLE AND COLCHICINE TREATMENT

Drug	Dose	Time drug applied (min) [a]	Water flow (J_v) [b] ($mg \cdot min^{-1} \cdot cm^{-2}$)
Nocodazole	0.0 (Control)	− 30	1.70 ± 0.32 (6)
Nocodazole	0.1 μg/ml	− 30	1.56 ± 0.33 (6)
Nocodazole	1.0 μg/ml	− 30	0.96 ± 0.23 (7)
Nocodazole	10.0 μg/ml	− 30	0.54 ± 0.23 (5)
Nocodazole	0.0 (Control)	− 60	1.49 ± 0.29 (5)
Nocodazole	10.0 μg/ml	− 60	0.55 ± 0.06 (5)
Colchicine	0.0 (Control)	−180	1.60 ± 0.20 (6)
Colchicine	0.1 mM	−180	0.78 ± 0.10 (6)
Nocodazole	0.0 (Control	+ 3	1.00 ± 0.10 (7) [c]
Nocodazole	1.0 μg/ml	+ 3	1.00 ± 0.10 (7) [c]

[a] Time before (−) or after (+) vasopressin (20 mU/ml) stimulation.

[b] J_v measured between 0 and 10 min after vasopressin stimulation except where noted.

[c] J_v measured between 20 and 20 min after vasopressin stimulation.

Results

Nocodazole, when applied 0.5 h prior to stimulation at levels known to disrupt microtubules [6,8], inhibited the vasopressin-induced increase in water permeability in toad bladder measured at 10 min following stimulation. Increasing inhibition was seen with the application of larger doses (Table I). The maximum dose, 10 μg/ml, gave an inhibition of 68% when applied 30 min prior to ADH stimulation and 63% when applied 1 h prior to ADH stimulation. Incubation times of less than 0.5 h show less than maximal inhibition (data not shown). Exposure of the tissue to colchicine at a concentration of 0.1 mM 3 h prior to hormonal stimulation brought about a 50% inhibition (Table I).

The rapid action of nocodazole allowed investigation of the involvement of microtubules during the half hour period following stimulation of toad bladder by vasopressin. Nocodazole was injected three minutes after stimulation to a final concentration of 1 μg/ml. Measurement of the permeability

0.5 h after stimulation shows no significant difference between the control and experimental chambers (Table I).

Discussion

Studies with the tubulin-binding drugs colchicine and nocodazole show very similar effects. Both of these agents, when applied prior to stimulation of the bladder with vasopressin, brought about an inhibition in the tissue's increase in permeability. Nocodazole is a synthetic antitumor drug that binds quickly and reversibly to tubulin [9]. Once introduced this drug quickly disrupts microtubules [6,8]. Nocodazole can rapidly disrupt microtubules without the side effects of colchicine indicated by Beebe et al. [6] and it also rapidly brings about an inhibition in the hydroosmotic response of toad bladder to vasopressin. This would indicate that the inhibition of this response brought about by colchicine is dependent upon its disruptive effects on microtubules and not on a membrane-associated side effect of this drug. The inhibition brought about by nocodazole further strengthens the arguement that an intact microtubule apparatus is necessary for the full increase in the transtissue water permeability of toad bladder.

The rapid action of nocodazole allows further investigation of the role of microtubules. In this case their involvement in the permeability change during the first half hour following hormonal stimulation. As has been shown, the addition of nocodazole after stimulation has no significant effect on the permeability increase measured at 0.5 h. This agrees with the work of Kachadorian et al. [2] who found that addition of colchicine to toad bladder one-half hour after vasopressin stimulation had no effect on water permeability observed 2.5 h later. Muller et al. [10], based on freeze-fracture studies in toad bladder, have suggested that microtubules are only needed during the first ten minutes after vasopressin stimulation. The results with nocodazole strengthen this conclusion since it shows that the need for intact microtubules is confined to the early stages of vasopressin stimulation and is over 0.5 h after stimulation. From this it is clear that intact microtubules are not needed for the maintenance of the permeability increase seen 0.5 h after stimulation.

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