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CALMODULIN ANTAGONIST INHIBITION OF POLYAMINE TRANSPORT IN PROSTATIC CANCER CELLS IN VITRO

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Cells stimulated to divide increase their uptake of extracellular polyamines by a transport system that is specific for the natural polyamines, such as putrescine, spermidine, spermine, and polyamine-like compounds (1). In the course of our experiments on characterizing the polyamine transport properties of PC-3 cells, we observed that the cationic peptide melittin was a potent inhibitor of polyamine uptake. As melittin is also one of the most potent of the reported antagonists of calmodulin, we investigated whether calmodulin antagonists would inhibit polyamine transport (2).

MATERIALS AND METHODS

The human prostatic cell line PC-3 was obtained from the Human Tumor Cell Laboratory of the Memorial Sloan-Kettering Cancer Center (MSKCC). The rat metastatic prostate cancer-derived MAT-LyLu cell line was obtained from Dr. John Isaacs of the Johns Hopkins School of Medicine. The calmodulin antagonists, trifluoperazine (TFP) and HPLC-purified melittin, and the polyamine putrescine were obtained from the Sigma Chemical Co., St. Louis, MO (2,3). 1,3-Dihydro-1-[1-(4-methyl-4H,6H-pyrrolo[1,2-9][4,1]-benzoxazepin-4-yl)methyl]-4-piperidinyl]-2H-benzimadazol-2-one (CGS 9343B) was obtained from Dr. J. Matthey of Ciba-Geigy, Summit, NJ (3). The protein kinase inhibitor, 1-(5-isoquinoline-sulfonyl)-2-methylpiperazine dihydrochloride (H-7), was purchased from Seikagaku America, Inc., St. Petersburg, FL (4). All tissue culture medium additives were obtained from the Media Preparation Facility, MSKCC. [^{3}H]Putrescine (28 Ci/mmol) was obtained from Amersham, Arlington Heights, IL.

[^{3}H]Putrescine uptake was performed similarly to that described previously (5). Briefly, 10^5 MAT-LyLu and 2×10^5 PC-3 cells were plated per well in Costar 12-well cluster plates. Two days later the medium was aspirated, the cells were rinsed with Hanks' balanced salt solution (HBSS), and the medium was replaced with 1 ml of serum-free uptake medium containing 5 μM putrescine (3.2 μCi [^{3}H]putrescine) with or without (vehicle only control) the presence of the listed concentrations of antagonist. Following incubation with [^{3}H]putrescine for 20 min at 37°, the medium was aspirated, the cells were rinsed four times with HBSS, the cells were solubilized with 0.1 N NaOH, and an aliquot was taken for scintillation counting. Another aliquot was brought to pH 7.0, and protein was determined by differential UV absorption spectrometry (6).

RESULTS AND DISCUSSION

Melittin, in concentrations ranging from 0.15 to 1.2 μM, significantly and increasingly inhibited putrescine transport from 35 to 70% (Table 1). Melittin is a calmodulin

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Table 1. Effects of kinase and calmodulin antagonists on polyamine uptake and cellular detachment of the human prostatic cell line PC-3

Treatment	Protein recovered[a] (μg)	[^{3}H]Putrescine uptake[b]
Control	131 ± 7	5.85 ± 0.29
H-7 (200 μM)	133 ± 10	6.32 ± 0.79
Control	108 ± 8	6.35 ± 0.40
CGS 9343B (6.25 μM)	105 ± 10	3.11 ± 0.31[c] (49%)
(25 μM	95 ± 8	1.92 ± 0.26[c] (30%)
(100 μM)	91 ± 10 (84%)	1.63 ± 0.45[c] (26%)
Control	154 ± 7	4.71 ± 0.24
TFP (3.1 μM)	164 ± 3	3.65 ± 0.24[c] (77%)
(6.2 μM)	163 ± 11	2.67 ± 0.12[c] (56%)
(12.5 μM)	157 ± 6	2.07 ± 0.3[c] (44%)
(25.0 μM)	78 ± 4[c] (50%)	1.82 ± 0.16[c] (39%)
Control	154 ± 10	5.38 ± 0.48
Melittin (0.15 μM)	154 ± 3	3.5 ± 0.22[c] (65%)
(0.3 μM)	96 ± 11[c] (62%)	2.3 ± 0.2[c] (43%)
(0.6 μM)	45 ± 4[c] (29%)	2.1 ± 0.17[c] (39%)
(1.2 μM)	32 ± 2[c] (21%)	1.6 ± 0.25[c] (30%)

[a]Mean μg protein per well ± SD, N = 4.

[b]Average putrescine uptake in pmol/100 μg protein/20 min ± SD, N = 4.

[c]Significantly different from control ($P < 0.01$) by Student's t-test; number in parentheses represents significant treatment effects as a percent of control.

antagonist (2). Calmodulin antagonists have been reported to decrease cellular adhesion to tissue culture plates (7). Therefore, we measured cell recovery and found that it correlated well with protein recovery, which is recorded in Table 1. At the lowest melittin concentration tested, no loss in recovery of protein was observed, but a significant 35% decrease in putrescine uptake was found. This decrease in putrescine uptake was more pronounced at higher concentrations of melittin. TFP is also a calmodulin antagonist (3). TFP treatment resulted in significant cell loss at a concentration of 25 μM. At lower concentrations of 3.1, 6.2, and 12.5 μM no significant decrease in cell attachment was observed but TFP increasingly inhibited putrescine uptake from 23 to 61%.

Calmodulin antagonists, such as TFP, have also been observed to inhibit protein kinase C (PKC) (3). The isoquinolinesulfonamide derivative H-7 is an inhibitor of cAMP and cGMP dependent protein kinases and a potent inhibitor of PKC (4). As it could be argued that TFP elicits its effect on putrescine transport through inhibition of PKC, we determined putrescine uptake at many concentrations (data not shown) of H-7. H-7, even at the highest concentration tested (200 μM), did not inhibit putrescine uptake. H-7 is not effective as a calmodulin antagonist (8), nor did it inhibit putrescine uptake. CGS 9343B is a calmodulin antagonist that is more specific than many of the other calmodulin antagonists and it does not inhibit PKC (3). Maximum inhibition of putrescine uptake was achieved at 25 μM CGS 9343B, while protein recovery was reduced by just 16% at 100 μM. The maximal inhibition of putrescine uptake of PC-3 cells by these calmodulin antagonists was 70%.

We also examined whether these calmodulin antagonists would reduce polyamine uptake in the rat prostate-derived R3327 MAT-LyLu cancer cell line (Table 2). CGS 9343B was much less inhibitory and only decreased uptake by 12% at 25 μM, compared with the 70% inhibition

Table 2. Effects of calmodulin antagonists on polyamine uptake in the rat prostatic cell line MAT-LyLu

Treatment	[^{3}H]Putrescine uptake[a]
Control	42 ± 2.5
CGS 9343B (25 μM)	37 ± 2.1 (88%)[b]
Control	37 ± 1.8
TFP (6.25 μM)	21 ± 0.7[c] (57%)
Control	51 ± 2.8
Melittin (0.5 μM)	26 ± 2.3[c] (52%)

[a]Values, expressed in pmol/100 μg protein/20 min, are means ± SD, N = 4.
[b]Numbers in parentheses represent percent of control.
[c]Significantly different from control by Student's t-test, P <0.01.

observed in PC-3 cells. An inhibition of 20% was found at 200 μM. TFP and melittin reduced uptake by 50% at concentrations which reduced putrescine uptake into PC-3 cells by 50%. It appears that MAT-LyLu cells are less sensitive to the calmodulin antagonist CGS 9343B, while equally sensitive to TFP and melittin with regard to putrescine uptake.

MAT-LyLu cells have a higher rate of polyamine uptake than PC-3 cells. The rate of putrescine uptake into PC-3 cells can be increased by decreasing intracellular polyamine content with α-difluoromethylornithine (DFMO), a specific polyamine synthesis inhibitor (5). A 5-fold increase was observed in the DFMO-treated cells (Table 3). The PKC inhibitor H-7

Table 3. Effects on putrescine uptake of intracellular polyamine depletion of PC-3 cells by the polyamine synthesis inhibitor DFMO and calmodulin antagonists

Treatment	Putrescine uptake[a]	
	(- DFMO)	(+ DFMO)[b]
Control	6.7 ± 0.5	33.4 ± 2.3
H-7 (50 μM)	6.2 ± 0.4 (92%)	34.7 ± 3.1 (104%)
CGS 9343B (5 μM)	3.5 ± 0.5[c] (56%)	12.6 ± 0.5[c] (38%)
TFP (6.2 μM)	4.1 ± 0.1[c] (61%)	10.4 ± 3.2[c] (31%)
Melittin (0.15 μM)	2.6 ± 0.2[c] (39%)	9.7 ± 0.7[c] (29%)

[a]Values, expressed in pmol/100 μg protein/20 min, are means ± SD, N = 4. Number in parentheses represents treatment as a percent of control.
[b]DFMO (1 mM) was included for 2 days following plating (5).
[c]Significantly different from control by Student's t-test, P <0.01.

did not alter polyamine uptake significantly. The percentage inhibition of putrescine uptake caused by the calmodulin antagonists was slightly greater in the DFMO-treated cells. Intracellular polyamine depletion and the resulting increase in putrescine uptake did not reduce the ability of the calmodulin antagonists to inhibit putrescine uptake.

In many experiments these calmodulin antagonists inhibited polyamine accumulation in these tumor cell lines. In this report, calmodulin antagonists have been shown to inhibit putrescine accumulation following a 20-min period of incubation. This would be the result of either a reduced rate of influx or enhanced rate of efflux or both. Kinetic experiments will be required to establish the nature of the inhibition of polyamine accumulation by

calmodulin antagonists. Studies with these inhibitors will be useful in gaining insight into the relationship between polyamine transport and calmodulin in cell growth and function (9-11).

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