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DANCYLCADAVERINE ELIMINATES CALMODULIN STIMULATION OF PHOSPHODIESTERASE

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Dansylcadaverine, which structurally resembles the calmodulin antagonists W-7 and W-5, prevented the calmodulin dependent stimulation of 3':5'-cyclic nucleotide phophodiesterase in vitro. Dansylcadaverine and trifluoperazine sensitized cells to Pseudomonas aeruginosa exotoxin A in apparently the same way, exept that 40 times higher concentrations of dansylcadaverine than of trifluoperazine was required.

Recently we found that several cell lines can be strongly sensitized to the action of Pseudomonas aeruginosa exotoxin A when treated with the antipsychotic drug trifluoperazine as well as with a variety of other calmodulin inhibitors (A. Sundan, K. Sandvig, S. Olsnes, manuscript submitted for publication). During this work we discovered that dansylcadaverine, which has been extensively used to inhibit receptor-mediated uptake of a variety of ligands (1), sensitized cells to the toxin in an analogous manner as trifluoperazine and other calmodulin antagonists. This suggested that dansylcadaverine inhibits calmodulin mediated processes.

MATERIALS AND METHODS

Bovine heart 3':5'- cyclic nucleotide phosphodiesterase (P-0520) was obtained from Sigma Chem.Co., St. Louis, Mo., alkaline phosphatase and calmodulin from Boehringer Mannheim GmbH, W-Germany, dansylcadaverine from Fluka A.G. Switzerland, and trifluoperazine from Théraplix A.S. Paris, France, amitriptylin from Lundbeck & Co A.S., Copenhagen, Denmark, and propranolol was from Sigma Chem. Co., St.Louis, Mo.

Cell culture and measurement of protein synthesis inhibition were carried out as described (2).

The Ca^{2+}/calmodulin activation of 3':5'- cyclic nucleotide phosphodiesterase was tested according to the method

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described by Sharma and Wang (3). In this system, the 3':5'-cyclic nucleotide phosphodiesterase is activated approximately four fold in the presence of saturating concentrations of Ca^{2+} and calmodulin. Half maximal activation in this system was obtained with 5 ng calmodulin.

RESULTS AND DISCUSSION

When Ca^{2+} and calmodulin were added to a cell-free assay for 3':5'-cyclic nucleotide phosphodiesterase (PDE) activity, the rate of the enzymatic reaction increased approximately four fold in the presence of optimal concentrations of calmodulin. When the calmodulin inhibitors trifluoperazine, amitriptyline, and propranolol (6,7) were added, the stimulation by calmodulin was eliminated (Fig. 1). Trifluoperazine inhibits calmodulin dependent reactions by binding to calmodulin (4). A stoichiometric ratio has been found between the calmodulin concentration in the assay system and the concentration of

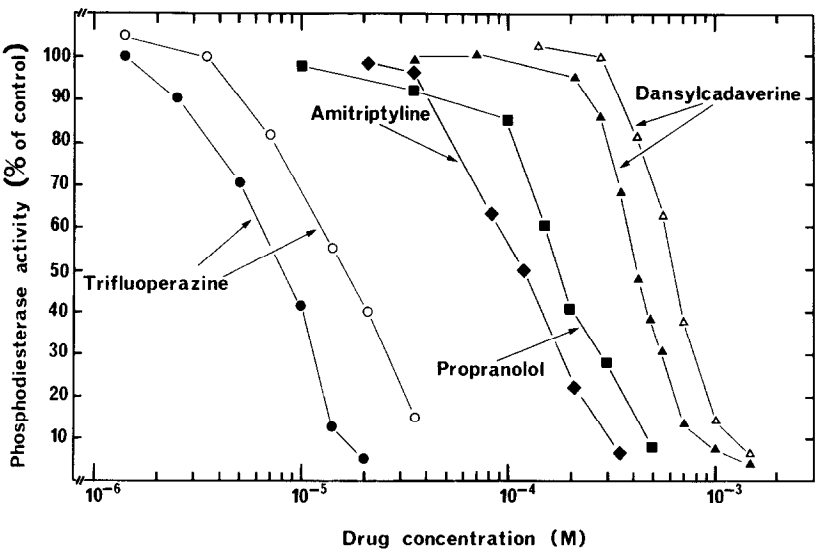

Fig.1. Ability of different compounds to counteract the stimulation of phosphodiesterase by calmodulin. 3':5'-cyclic nucleotide phosphodiesterase was assayed in the presence of 20 ng calmodulin (closed symbols) or 40 ng calmodulin (open symbols) as described under Materials and Methods. In each case control assays without calmodulin present as well as assays containing 40 ng calmodulin and 0.2 mM EGTA (ethyleneglycol-bis-(β-aminoethyl ether) N,N -tetra-acetic acid) were carried out. The amount of phosphate liberated in these assays was subtracted before the experimental data were plotted.

trifluoperazine required for inhibition (5). In accordance with this we found that whereas 8 μM trifluoperazine was sufficient to reduce by 50 % the stimulation obtained with 20 ng calmodulin, 15 μM trifluoperazine was required when the concentration of calmodulin was doubled (Fig. 1). Amitriptyline and propranolol inhibited the activity of the stimulated phosphodiesterase by 50 % at concentrations of 120 μM and 175 μM, respectively, in accordance with earlier reports (6,7).

The significant finding in Fig. 1 is that also dansylcadaverine inhibited the reaction in concentrations close to those required for amitriptyline and propranolol. The activity of the phosphodiesterase stimulated with 20 ng calmodulin was reduced by 50% in the precence of 410 μM dansylcadaverine , while 640 μM dansylcadaverine was required to obtain the same effect in the presence of 40 ng calmodulin.

To compare the effect of trifluoperazine and dansylcadaverine in an in vivo assay, we took advantage of our recent observation that all inhibitors of calmodulin tested, including dansylcadaverine, strongly increased the sensitivity of BHK cells to Pseudomonas aeruginosa exotoxin A. This is illustrated in Fig. 2 which shows that with increasing concentrations of trifluoperazine in the cell culture medium, the concentration of toxin required to reduce cellular protein synthesis to half the control value (ID_{50}) was reduced. Dansylcadaverine sensitized the cells to approximately the same extent.From the data in Fig. 2 the concentration of trifluoperazine giving half maximal sensitization can be estimated to be about 1 μM, while 40 μM dansylcadaverine was required to obtain the same effect. This 40 fold difference in concentrations required in vivo compares favourably with the 50 fold difference found in the in vitro test system in Fig. 1.

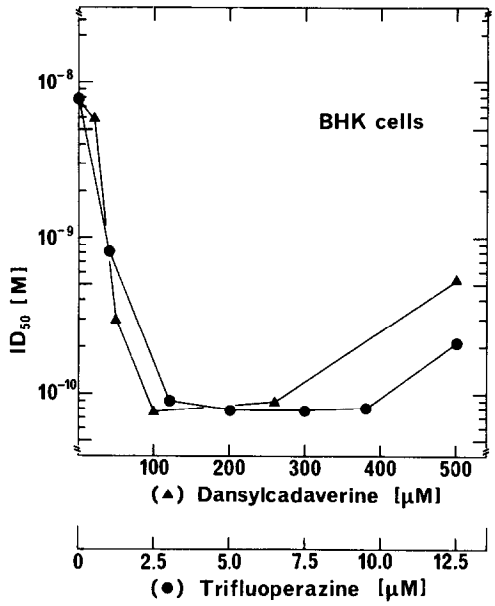

Fig.2. Ability of trifluoperazine and dansylcadaverine to sensitize BHK cells to exotoxin A from Pseudomonas aeruginosa. BHK cells were preincubated with the indicated concentrations of trifluoperazine and dansylcadaverine for 15 min at 37 °C and then increasing concentrations of toxin were added. After 3 h the medium was removed and leucine-free medium containing 1 uCi/ml of ^3H leucine was added. The incorporation of radioactivity during a 10 min interval was measured. At each concentration of the drugs tested the toxin concentration required to reduce protein synthesis to half the control value (ID_{50}) was calculated and these values were then plotted against the concentration of trifluoperazine (●); and dansylcadaverine (▲).

Dansylcadaverine has been extensively used in studies of receptor-mediated endocytosis. It has been shown to inhibit the clustering into coated pits of α_2-macroglobulin and epidermal growth factor (1). Since dansylcadaverine is a strong inhibitor of transglutaminase, it was suggested that this enzyme could play a role in receptor-mediated endocytosis. Here we show that dansylcadaverine inhibits calmodulin stimulated PDE. Since the recruitment of clathrin to coated pits in the plasma membrane is dependant on calmodulin (8), the possibility should be considered that the observed effect of dansylcadaverine on the clustering of epidermal growth factor and α_2-macroglobulin (1) is mediated by its effect on calmodulin.

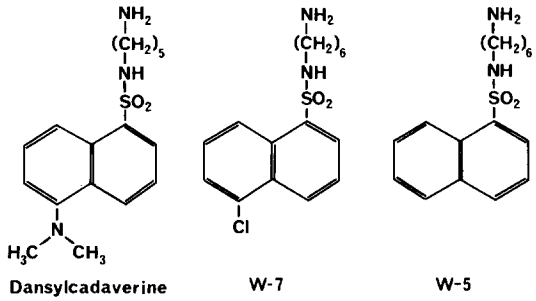

Fig.3. Structure of dansylcadaverine (N-(5-aminopentyl)-5-dimethylamino-1-naphthalenesulfonamide), W-7 (N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide) and W-5 (N-(5-aminohexyl)-1-naphthalenesulfonamide).

In fact Haigler et al. (9) found that chlorpromazine, which is also a potent calmodulin antagonist, inhibited the internalization of epidermal growth factor. Chlorpromazine sensitizes cells to Pseudomonas aeruginosa exotoxin A in the same way as trifluoperazine (our unpublished data).

Hidaka et al. (10) recently described two new calmodulin antagonists termed W-5 and W-7. The most potent of these, W-7, is N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide while W-5 lacks Cl in position 5. The structure of these compounds and that of dancylcadaverine are shown in Fig.3. It is clear that the structures of the three compounds are strikingly similar.

Several similar naphthalenesulfonamide derivatives were also tested in Hidakas laboratory with respect to inhibition of calmodulin (7). Manipulations with the side chain in position 5 altered the potency of the compound as calmodulin antagonist, but considerable alterations could be carried out without complete loss of activity. It is therefore not surprising that also dansylcadaverine acts as a calmodulin antagonist.

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