## Inhibition of cAMP Phosphodiesterase by Some Phototherapeutic Agents

Lucia Bovalini, Paola Lusini, Sandra Simoni*, Daniela Vedaldi**, Lucio Andreassi*, Francesco Dall'Acqua**, and Paola Martelli

Institute of Biological Chemistry and Department of Dermatology*, University of Siena, Italy, and Department of Pharmaceutical Sciences**, University of Padova, Italy

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The behaviour of cyclic-3′,5′-AMP phosphodiesterase has been studied in the presence of psoralen, 8-methoxypsoralen (8-MOP), 4,5′,8-trimethylpsoralen (TMP) (usually used in PUVA therapy), 4,6,4′-trimethylangelicin (TMA) and khellin recently proposed for the same therapeutical use. TMP and TMA exhibit a significant inhibitory effect on cyclic AMP phosphodiesterase; a light inhibition is produced by khellin at rather high concentration.

### Introduction

The influence of dihydropyrano- and dihydrofuranocoumarins on the cyclic 3′,5′-AMP (cAMP) level in guinea-pig myocardial tissue and on the activity of purified beef heart cAMP phosphodiesterase (cAMP-PDE) [EC 3.1.4.17] is described in the literature [1]; results on the topic show that the coronary vasodilatatory activity is correlated both with the increase of cAMP level and the inhibition of cAMP-PDE.

In this study we report the results on the behaviour of cAMP-PDE from rat liver in the presence of psoralen, 8-methoxypsoralen (8-MOP), 4,5′,8-trimethylpsoralen (TMP) (furocoumarins usually employed in the PUVA therapy of some skin diseases), angelicin (reference compound of angular furocoumarins), 4,6,4′-trimethylangelicin (TMA) and khellin, a natural furanochromone contained in *Amni visnaga* plant. The two last compounds are phototherapeutic agents under clinical investigation [2–7].

Reprint requests to Prof. L. Bovalini, Istituto di Chimica Biologica, Pian dei Mantellini, 44, I-53100 Siena, Italy.

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### Results

As reported in the Table, psoralen, angelicin, 8-MOP and khellin doesn't significantly affect cAMP-PDE at a concentration of $1 \times 10^{-4}$ M; at the same concentration TMA and TMP exhibit an approximate inhibition of 40–45% statistically significant for $p < 0.01$. Preliminary results indicate that at lower concentration ($1 \times 10^{-5}$ M) only TMA and TMP exhibit a light inhibition value of about 20%. The cAMP-PDE has an approximate inhibition of 15% in the presence of $1 \times 10^{-3}$ M khellin (proposed for topical use at rather high concentration) [6]. The exposure of the assay mixtures to the UV-A light of 365 nm (1 J/cm$^2$) doesn't significantly affect the results obtained.

The study is worth completion. Future researches however should be made considering that cAMP-PDE is described as a group of isoenzymes that exhibits multiple forms with different substrate specificities and kinetic properties: therefore, we consider it useful, especially in respect to the therapy of psoriasis, to study the behaviour of the furocoumarins (or drug structurally related) towards epidermal cAMP-PDE. Infact literature data [8] report that in the complex pathophysiology of psoriasis a defective cAMP cascade appears to be a very important factor: the most likely sites for this metabolic defect are at cell surface, adenylate cyclase complex or at level of degradation of cAMP by PDE. Obviously a phototherapeutic agent exhibiting an enhanced inhibitory effect on the skin cAMP-PDE would be a more effective drug than a compound deprived of this property.

Table. Effect of khellin and furocoumarins on cAMP phosphodiesterase activity.

| Chemicals[a] | Number of rats/ experiments | nmol/min/ mg pr.[b] | S.D. | p |
|---|---|---|---|---|
| Control | 3 | 16.03 | ± 3.96 | – |
| Psoralen | 4 | 15.38 | ± 2.31 | n.s. |
| 8-MOP | 4 | 12.97 | ± 3.05 | n.s. |
| TMP | 4 | 9.76 | ± 3.48 | < 0.01 |
| Khellin | 4 | 12.40 | ± 2.18 | n.s. |
| TMA | 4 | 10.51 | ± 2.14 | < 0.01 |
| Angelicin | 4 | 14.06 | ± 2.87 | n.s. |

[a] The concentration of chemicals was $1 \times 10^{-4}$ M.
[b] The values were the average of triplicates from 3 separate experiments.

## Experimental

*Materials*

cAMP Kit was from Amersham Radiochemical Center (Amersham, England), cAMP was from Boehringer (Boehringer Mannheim, Germany), psoralen and angelicin from Franco Indian Company (Bombay, India), 8-MOP from Chinoin SpA (Milano, Italy), TMP from Sigma Chemical Company (St. Louis, MO, USA), khellin from Fluka (Buchs, Switzerland); TMA was prepared according to reference [3].

*Preparation of liver homogenate*

Wistar rats weighing approximately 180 g, fed on a normal mixed laboratory diet, were used in this study. The rats were killed by cervical dislocation and the liver was immediately homogenized in 1:5 ratio (w/v) in 50 mM Tris-HCl buffer pH 7.6. The homogenate was centrifuged at $20{,}000 \times g \times 20$ min at 4 °C in a ALC 972R centrifuge. The precipitate was discarded and the supernatant resuspended to the original volume with the same buffer used for the homogenization. The fraction was prepared just before use.

*Preparation of chemical solution*

Chemical solutions were prepared at concentration $3.3 \times 10^{-4}$ M containing 1% ethanol.

*Assay of cAMP-PDE activity*

The enzymatic activity was assayed in an incubation mixture of 0.2 ml containing 50 mM Tris-HCl pH 7.6, 10 mM $MgCl_2$, 1 mM cAMP and khellin or psoralens tested at final concentration of $1 \times 10^{-4}$ M. The reaction was started by adding a supernatant equivalent to 4 mg of fresh tissue. Successive operations were carried out using the kit Amersham as described by Lusini and Ricci [9]. The ethanol concentration present in the assay mixture doesn't affect enzymatic activity. Proteins were determined by Lowry method as reported in [9]. Enzymatic unit is expressed as nmol of cAMP hydrolyzed/min/mg protein. When required, the assay mixtures were exposed to UV-A radiation using a lamp HPW 125 W Philips; the irradiance of the emitted light was measured with an OSRAM UV-Meter. The radioactivity was measured with a Searle Nuclear Chicago Delta 300 Liquid Scintillation Counter.

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