Inhibition of cAMP Phosphodiesterase by Some Phototherapeutic Agents

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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].

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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 1×10^{-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 (1×10^{-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 1×10^{-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 1×10^{-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 3.3×10^{-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 1×10^{-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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