Effect of GABA, its analogs and antagonists on corneal deturgescence*

Material	Concentration (μM)	No. of experiments	Final thickness (μm)	Survival time (h)
1) Salt solution***		4	480±5	2.5±0.3
2) 1+oxydised glutathione	100	6	460±5	3.5±0.2
3) 1+adenosine	5000	15	400±5	6.0±0.3
4) 1+GABA	1	4	410±4	3.5±0.2
5) 1+GABA	10	5	390±7	6.2±0.3
6) 1+muscimol	10	4	385±6	6.0±0.3
7) 6+bicuculline	≃50**	4	480±6	2.0±0.2
8) 5+chlorpromazine	5	4	480±6	3.0±0.2
9) 5+picrotoxin	50	4	400±4	5.0±0.2
10) 1+glycine	100	4	460±5	3.0±0.2

* The method of study used is described by Dikstein and Maurice[5]. We used the modification of Anderson et al.[6]. ** Saturated solution. *** Does not contain glucose.

of 5 mM adenosine - an unphysiological concentration - the deturgescence reaches near completion and the survival time is increased to 6 h[5-7].

Many metabolites were tested unsuccessfully, including sugars, glycolytic intermediates, Krebs cycle intermediates, agents influencing c-AMP formation and prostaglandins[8], with the aim of finding the natural agent permitting full temperature reversal.

We wish to report that GABA, in the range of 10 μM, causes complete reversal with a survival time of 6 h. The GABA analog muscimol is about as active as GABA (table). The action of GABA cannot effectively be blocked by picrotoxin, which in other preparations blocks the chloride ionophore of the receptor[9] in line with the lack of importance of chloride ions for the corneal endothelial fluid pump[10]. On the other hand, the effect of GABA and muscimol is blocked by chlorpromazine and bicuculline respectively in line with the known blocking effect of these agents on the target area of the receptor[11]. Glycine at much higher concentrations than GABA had no effect.

The importance of our finding is: a) It provides an easily and continuously monitorable, single layer uniform cell preparation, in which the receptor stimulated fluid transport can be conveniently studied. Indeed the advantages of this preparation over others have already been pointed out[10,12]. b) It shows that GABA might have an important function outside the nervous or invertebrate neuromuscular systems. c) It increases the chance of developing a long-term preserving fluid for corneal transplantation.

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λ-Prophage induction by furocoumarin photosensitization

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Summary. Furocoumarin photosensitization induces λ-prophage from lysogenic *Escherichia coli* cells; this effect is clearly due to the photoreaction that furocoumarins give with DNA, and it appears connected with the formation of monoadducts rather than of diadducts (cross-links).

Photosensitizing furocoumarins are a group of natural or synthetic drugs which, by irradiation with long wave UV-light, are able to link covalently to the pyrimidine bases of DNA[2]. Furocoumarins having an angular molecular structure, such as angelicin derivatives, behave as monofunctional reagents, forming only monoadducts (1 furocoumarin molecule + 1 base molecule)[3]. Linear furocoumarins, i.e. psoralen derivatives, form both monoadducts and diadducts (1 furocoumarin molecule + 2 base molecules), giving in this latter case interstrand cross-links[4,5].

The biological consequences of such damage has been studied by several authors on very different substrates, in such a manner that a high number of biological effects of furocoumarin photosensitization are now known. We remember the erythemas on human or guinea-pig skin[2], the killing[6] and the mutations[7] of bacteria, or of mammalian cells grown in vitro[8], the loss of the tumor-transmitting capacity for experimental tumors of the mouse[9,10], the inactivation of some DNA viruses[11] and so on.

In this paper we report on a new effect of photosensitizing furocoumarins, that is the induction of the λ-prophage from lysogenic *E. coli* cells. Lambda is the most studied among the temperate phages, which, upon infection, insert their DNA into the host chromosome, producing a new bacterial strain (lysogen), carrying the virus genome (prophage). These virus genes, generally inactive, can be induced, with production of new infectious phage particles, by serious damage afforded to bacterial DNA[12].

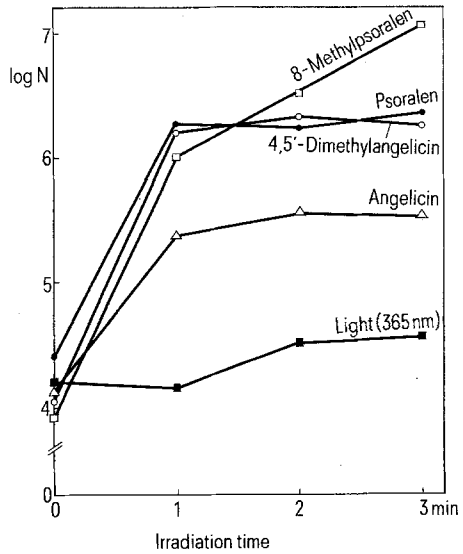
E. coli K_{12} bacteria (Hfr 1/2 + E; Sms streptomycin sensitive, carrying the λ-prophage) were grown in brain heart infusion (Difco Laboratories), collected in log phase and suspended in physiological saline (10^8 cells/ml), containing the examined furocoumarin. Cells were kept in the dark at room temperature for 15 min and then irradiated in Petri dishes (5 cm in diameter), placed on crushed ice, by means of a Philips HPW 125 lamp provided with a Philips filter, set at 50 cm distance (365 nm; irradiation intensity incident on the whole sample: 2×10^{17} quanta/sec). In these experimental conditions, using a drug concentration of 4 µg/ml and irradiation times below 10 min, no significant decrease of the surviving fraction of the irradiated lysogenic bacteria, i.e. the colony-forming capacity on agar plates, was observed. The irradiated lysogens were plated on dishes prepared with brain heart infusion agar, using the bilayer method[13] and *E. coli* K_{12} S, Smr streptomycin resistent as indicator bacteria. After 2 h at 37 °C, a 2%-solution of streptomycin sulphate was sprayed on plates, that were further incubated overnight; the number of the induced bacterial cells per ml, i.e. the plaque-forming units, was then determined[14].

The figure shows the results obtained with some typical photosensitizing furocoumarins; as already found with other biological systems, the photosensitized effect can be observed only after irradiation in the presence of the drug. Actually, incubation in the dark in the presence of furocoumarins was not effective, while long wave UV-light proved to be a very poor inducer. The number of the induced cells was small for angelicin, high for psoralen and 4,5′-dimethylangelicin and very high for 8-methylpsoralen; this result is consistent with the different photoreactivity of the furocoumarins studied, i.e. the ability to link covalently to DNA, without distinction between the different types of adducts that they form in DNA[2].

Moreover, from these data no correlation between λ-prophage induction and the capacity to form cross-links in DNA is evident; in fact while angelicin and 4,5′-dimethylangelicin are not able to induce this damage, psoralen and 8-methylpsoralen are very active bifunctional drugs. This fact is more evident when we compare the results obtained with psoralen and 4,5′-dimethylangelicin. This last compound is a new monofunctional furocoumarin, showing a high photoreactivity of practically the same degree as psoralen[15,16]. Thus, lysogenic bacteria irradiated in the presence of the same concentration of these 2 drugs contained the same number of furocoumarin moieties linked to their DNA, but only DNA of cells photosensitized by psoralen was damaged by cross-links. As shown in the figure, no significant difference between the inducing abilities of the 2 drugs was observed.

This new photosensitized effect is clearly connected with the photoreaction that furocoumarins give with bacterial DNA. In fact, λ-prophage induction must be correlated to a persistent damage to chromosomal DNA, or to an intervention of an error-prone DNA repair such as the so-called SOS repair[17]. Both monoadducts and cross-links induced by furocoumarins in DNA can be repaired, even if by very different mechanisms. Cross-link repair, in particular, requires genetic recombination[18], and if the number of the repaired cross-links is not small, it yields a biologically inactive DNA, which produces cell death[19]; in fact, several authors have correlated the lethal effect of furocoumarin photosensitization on cross-link formation[6-8,19]. Monoadducts can also induce cell death, although with lower ability[3], but they seem connected with the mutagenic activity of furocoumarins[7,20].

On the basis of these preliminary data, λ-prophage induction appears in same way connected with monoadducts rather than with cross-links, at least in our experimental conditions in which very low radiation doses were used; further research is now in progress in connection with this question.



λ-Prophage induction by furocoumarin photosensitization. Lysogenic bacteria (10^8/ml) were irradiated at 365 nm in the presence of 4 µg/ml of furocoumarin and the induced cells were scored. The irradiation time was plotted against the log of the number of the induced cells per ml (log N). No significant induction was observed by incubating lysogens in the dark in the presence of furocoumarins; the line (■——■) was obtained by irradiating cells in the absence of the drugs. For comparison, lysogenic bacteria were irradiated by a germicidal Philips TUV-15W lamp, placed at 50 cm distance; after 3 min irradiation, a 6.2 value of log N was obtained.

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