INHIBITION OF IN VITRO HISTONE ACETYLATION IN A CELL FREE RAT UTERINE SYSTEM BY METHYLGLYOXAL AND PHENYLGLYOXAL

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Recently, much interest has been devoted to the biological activity of glyoxal derivatives. In 1967, Szent-Gyorgi proposed that these compounds may have a growth inhibitory function in biological systems. Interestingly, methylglyoxal and propylglyoxal were shown to inhibit cell division in mouse lymphoma cells (Gregg, 1968). Scaife (1969) has presented evidence that two hetoaldehydes, methylglyoxal and β-ethoxy α-hetobutyraldehyde (Kethoxal, UpJohn Pharmaceutical) inhibit cell division and protein synthesis in human kidney cells.

Concomitantly, a growing body of observations (Allfrey et al, 1963; Pogo et al, 1966, 1968) indicates that histone metabolism, in particular, histone acetylation, may be involved in the regulation of DNA function, since the inhibitory effect of histones is decreased by acetylation (Allfrey et al, 1964). Hence, the inhibitory action of histone may be regulated, in part, by the acetylation reaction (Georgiev, 1969). It follows that interference with the acetylation of histones may lead to an alteration in their overall function. In light of these findings, it was of interest to study the effects of glyoxal derivatives on in vitro histone acetylation and to ascertain the existence of a link between the activity of these proposed regulators. Results presented in

this paper indicate that methylglyoxal and phenylglyoxal influence histone metabolism in vitro by blocking histone acetylation. In addition, I_{50} values (approximate concentration at which 50% inhibition is observed) for both drugs are reported.

MATERIALS AND METHODS

Uterine acetylase enzyme, obtained from mature Sprague-Dawley rats (250 - 300g), was prepared and incubated according to Libby (1968). Phenylglyoxal (Pfalz and Bauer) and methylglyoxal (Aldrich Chemical) were dissolved in 0.5M phosphate buffer, pH 7.7 prior to use. Arginine-rich histone (Worthington Biochemicals) was used for all assays. Drugs were preincubated with enzyme for 15 min. at 37°C prior to addition of histone and the reaction was allowed to proceed for an additional 30 minutes. The acetylated histones were then extracted, precipitated, and dissolved in 0.05M barbital buffer, pH 9.0, containing 7.5M urea (Libby, 1968). One ml of the solution was transferred to scintillation vials containing 15 ml of toluene scintillation solution which was prepared by dissolving 4g 2-5-diphenyloxazole plus 50 mg of 1,4-phenylene bis [2-(5-phenyoxazole)] in 700 ml of toluene plus 300 ml of BBS-3 (Bio-Solv, Beckman Instruments, Inc.). The vials were counted in a Packard Tri-Carb Liquid Scintillation Spectrometer at a counting efficiency of approximately 70%

RESULTS AND DISCUSSION

The glyoxal derivatives, methylglyoxal and phenylglyoxal, inhibited the in vitro acetylation of calf thymus histone by a cell free preparation from rat uterus. The degree of inhibition was dependent upon the dose administered (Fig. 1). Phenylglyoxal proved to be slightly more potent than its methyl congener, as reflected by a lower I_{50} value (Table 1). Glyoxals have been

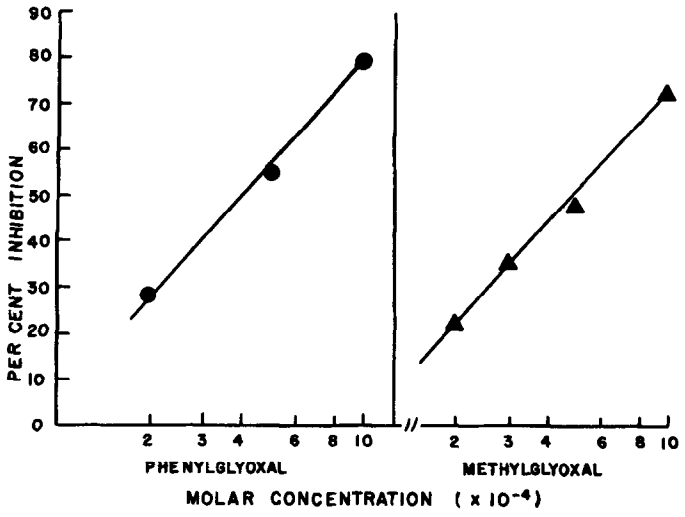

Fig. 1 The effect of Phenylglyoxal and Methylglyoxal on the in vitro acetylation of histones by a cell-free rat uterine system. Freshly prepared solutions of glyoxals were added at various concentrations and pre-incubated for 15 minutes at 37°C prior to the addition of histone. The reaction was allowed to proceed for an additional 30 minutes. Each point represents at least three determinations.

TABLE I

INHIBITION OF IN VITRO HISTONE ACETYLATION BY GLYOXAL DERIVATIVES: INDEX OF INHIBITION (I_{50} VALUE)[a]

Compound	I_{50} Value
Methylglyoxal	0.5 mM
Phenylglyoxal	0.4 mM

[a] The drug concentration at which 50 per cent inhibition is observed. Various concentrations of inhibitors were preincubated for 15 min at 37°C prior to addition of 1 mg calf thymus histone (see Materials and Methods). The data was plotted and I_{50} values determined graphically.

shown to exist in various tissues at relatively high levels (Szent-Gyorgyi, 1967). This fact may account for the apparent high concentrations of glyoxal derivatives required to inhibit histone acetylation, if these compounds are indeed capable of affecting histone metabolism in vivo as well as in vitro.

The function of glyoxals as cell growth regulators has been proposed (Szent-Gyorgyi, 1967). Interestingly, two glyoxal derivatives, methylglyoxal-bis-guanylhydrazone (Methyl-GAG) and β-ethoxy-α-Ketobutyraldehyde (Kethoxal) have found limited use in the treatment of leukemia (Freireich et al, 1962; French and Freelander, 1958). However, the exact mechanism of action of these drugs is not known (Henderson, 1969).

Conclusions to be drawn from the observations presented here are necessarily limited, however results do indicate that the cellular inhibitory properties of glyoxals may result, at least in part, from their ability to interfere with normal histone metabolism. This belief is strengthened by the fact that methyl GAG and Kethoxal, are capable of inhibiting in vitro histone acetylation in the system described in the text (Procaccini, unpublished).

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