HISTONE PHOSPHORYLATION IN THE REGENERATING RAT LIVER INDEPENDENT OF DIURNAL RHYTHMICITY

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1. Introduction

Recently published results from our laboratory [1] demonstrated a circadian rhythmicity in the phosphorylation of histones in the normal rat liver. This time factor could also influence histone phosphorylation in the regenerating liver. There are, however, no data in the literature taking this into consideration and conflicting results [2–7] could be attributable to these circumstances.

Our experiments differ from those described by other authors in that each hepatectomized animal was correlated with a simultaneously sham operated control animal. Under these conditions maximum phosphorylation of individual histone fractions was found 1, 2, 6, or 12 hr after partial hepatectomy. In addition, a general stimulation occurred 22 to 26 hr after the operation. Administration of 3′,5′-adenosine monophosphate occasionally resulted in further stimulations.

2. Methods

Male Sprague-Dawley rats, 3 months old, were hepatectomized according to Higgins and Anderson [8] by removal of 2/3 of their liver. Simultaneously, control animals were sham-operated by laparatomy under otherwise identical conditions. Both groups received 1 ml [^{32}P]orthophosphate (1.8 mCi, dissolved in 0.9% NaCl, containing 16.1 μmole of inactive orthophosphate per ml) 1 hr prior to sacrifice at the times indicated in fig. 1 and table 1. Livers were excised and histones were prepared, separated on 15% polyacrylamide gels, and stained as described previously [1]. Radioactivity of gel slices, solubilized with 0.2 ml 30% H_2O_2, was determined in a Packard liquid scintillation spectrometer in Bray's solution.

3. Results and discussion

The first stimulation of phosphate incorporation occurred 1 (F1′, F2a1), 2 (F1″, F2b, F3, F2a2) or 6 hr (F1) after partial hepatectomy. A second maximum was observed in some fractions 12 hr after the operation (only fractions F2a1, F2a2, F1′), whereas 22–30 hr after hepatectomy a general stimulation, affecting all histone fractions, was observed (fig. 1).

Although evidence is accumulating that direct and specific stimulation of transcription is more probably correlated with the phosphorylation of non-histone proteins of the cell nucleus, chemical modification of the histones is also involved in the regulation of genetic expression. The variety of phosphorylation maxima in the fractions of the F1 group, as demonstrated in fig. 1, is particularly striking. These fractions, which presumably differ only slightly in structure [9–11], have been suggested to be of importance in the condensation of heterochromatin in differentiated cell nuclei [12–15], whereas the arginine-rich fractions have been reported to play a primary role in the repression of transcription by interaction with RNA-polymerase [16]. One could speculate, therefore, that each phosphorylation maximum in the F2a, F2b, and F3 fractions requires a corresponding maximum in F1 group, thus correlating derepression and decondensation reactions. This could be an interpretation of the results shown in fig. 1.

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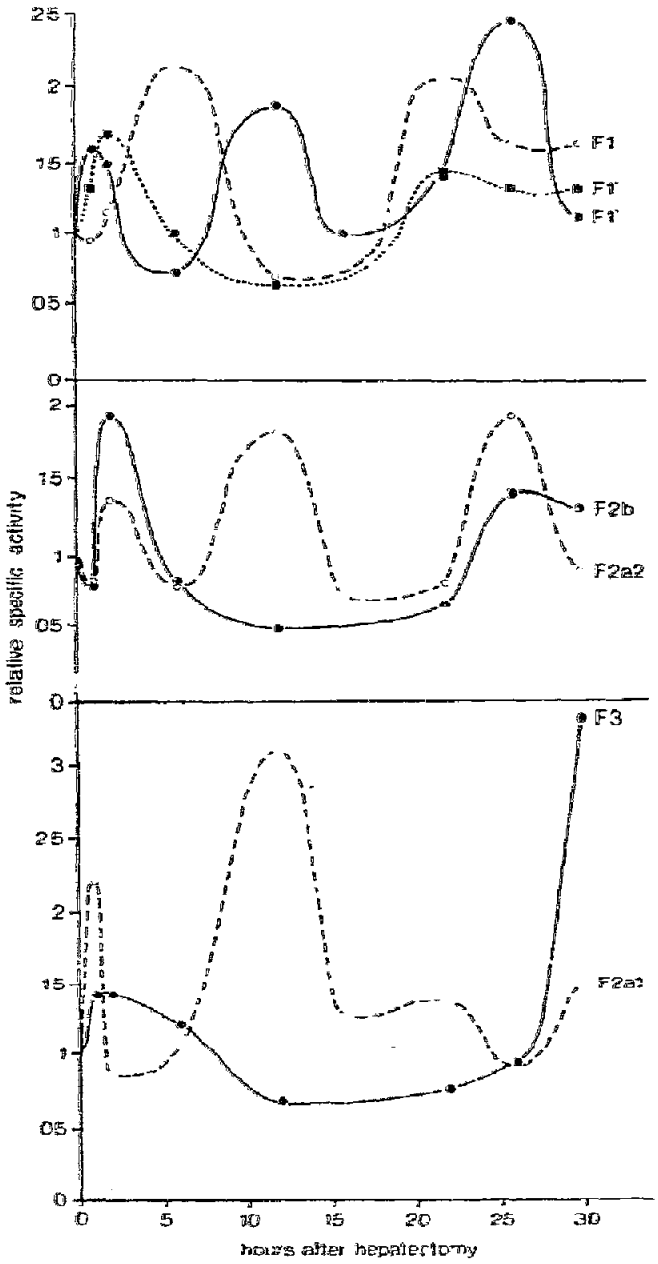

Fig. 1. Relative specific activities of individual histone fractions isolated at various times after hepatectomy from regenerating rat liver and separated on 15% polyacrylamide gels. Specific activities were obtained by dividing radioactivities of peak fractions after gel separation, by absorption at 620 nm of corresponding fractions from gels which had been stained with 0.1% amido black and which were scanned in a Chromoscan. Relative specific activity is

$$\frac{\text{specific activity regenerating liver}}{\text{specific activity control liver}}$$

Table 1

Stimulation of the phosphorylation of individual histone fractions at various times after hepatectomy by 3',5'-AMP.

Hr after hepatectomy	Histone fraction F1	F1'	F1''	F3	F2b	F2a2	F2a1
2	122	100	140	109	138	106	117
	112	72	105	83	106	123	100
6	168	141	106	88	80	206	133
	167	165	108	100	65	153	116
12	80	65	96	93	100	186	100
	70	59	104	100	75	290	88
22	166	155	143	140	93	91	87
	129	130	157	118	100	93	109
26	105	191	244	216	137	154	112
	113	165	185	307	133	160	89

The results of 2 experiments are given as per cent specific activity of 3',5'-AMP treated animals as compared to untreated animals. Rats were hepatectomized and were injected intraperitoneally with 1 ml (1.8 mCi) [^{32}P]orthophosphate and 1 ml 3',5'-AMP (pH 7.2, dissolved in saline) or saline 1 hr prior to decapitation.

Stimulation of F1 phosphorylation *in vivo* by external 3',5'-adenosine monophosphate (3',5'-AMP) has been described recently by Langan [17] and is confirmed by the present results. Furthermore, it is demonstrated that phosphorylation of other histone compounds is also stimulated by 3',5'-AMP (table 1). This is observed particularly in the phosphorylation of all F1 subfractions, as well as for fractions F3 and F2a2. Interestingly, an elevation of the endogenous 3',5'-AMP concentration in the liver 2.5 and 12 hr after hepatectomy has been reported by MacManus et al. [18]. With the exception of histone F2a2 no influence of 3',5'-AMP injections on specific activity was found under our experimental conditions for the 12 hr interval. Similarly, only limited stimulation occurred 2 hr after hepatectomy. This is presumably due to the fact that maximum stimulation has already been achieved by endogenous 3',5'-AMP concentrations. Six and 22–26 hr after hepatectomy, however, marked stimulations of the phosphorylation rate of most of the histone fractions could be observed.

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