Induction of rat α_2-macroglobulin in vivo and in hepatocyte primary cultures: synergistic action of glucocorticoids and a Kupffer cell-derived factor

Joachim Bauer, Manfred Birmelin, Gerd-Hinrich Northoff°, Wolgang Northemann, Thuy-Anh Tran-Thi, Heinz Ueberberg+, Karl Decker and Peter C. Heinrich*

Biochemisches Institut, Universität Freiburg, Hermann-Herder-Str. 7, D-7800 Freiburg, °Blutspendezentrale des DRK, Oberer Eselsberg, D-7900 Ulm, and *+Dr. Karl Thomae GmbH, Birkendorfer-Str. 65, D-7950 Biberach, FRG*

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Turpentine injection into rats elicits enhanced secretion of acute phase proteins inclucing α_2-macroglobulin (α_2M). Hypophysectomized rats, however, do not respond in this way unless dexamethasone is given together with turpentine. On the other hand, dexamethasone injection alone did not result in an induction of α_2M synthesis. When a medium of Kupffer cell cultures was added to hepatocytes, a dose-dependent stimulation of α_2M synthesis of up to 4-fold after 10–12 h was observed. However, the presence of low concentrations (10^{-9} M) of dexamethasone was essential for the stimulatory effect. We conclude that the acute phase induction of α_2M in hepatocytes requires the synergistic action of glucocorticoids and a non-dialysable factor secreted by Kupffer cells.

Acute-phase protein *α_2-Macroglobulin* *Glucocorticoid* *Hepatocyte-stimulating factor* *Kupffer cell*

1. INTRODUCTION

During inflammation the concentration of a group of plasma proteins – designated as acute phase proteins – is markedly increased (review [1–3]). In the rat these proteins consist mainly of proteinase inhibitors, such as α_2M, α_1PI, α_1APG and AT III. In previous studies we have shown that during experimental inflammation the levels of translatable liver mRNA for α_2M, TF, α_1AGP, α_1PI and α_1APG increase [4,5]. Similar results of experiments on the induction of mRNA for α_1AGP [6], fibrinogen [7], α_1PI [8,9], serum amyloid A [10], and haptoglobin [11] have been reported.

* To whom correspondence should be addressed

Abbreviations: α_2M, α_2-macroglobulin; α_1AGP, α_1-acid glycoprotein; AT III, antithrombin III; α_1APG, α_1-acute-phase globulin; α_1PI, α_1-proteinase inhibitor; TF, transferrin; LPS, lipopolysaccharide; SDS–PAGE, SDS-polyacrylamide gel electrophoresis

Numerous studies have dealt with the role of glucocorticoids in the acute phase response [12–16]. We have demonstrated that in rat hepatocyte primary cultures α_2M and α_1AGP can be induced by dexamethasone [17]. In addition, factors from leukocytes have been described as inducing acute phase protein synthesis [18–24] including interleukin 1 [25–27]. Recently Fuller and Wolosky (personal communication) found a polypeptide derived from human monocytes or rat Kupffer cells capable of stimulating fibrinogen synthesis in rat hepatocytes. This polypeptide has been designated as hepatocyte stimulating factor (HSF) by these authors.

Here we describe the induction of α_2M in rat hepatocyte primary cultures by a factor secreted by rat Kupffer cells. The action of this factor required the presence of glucocorticoids.

2. MATERIALS AND METHODS

L-[^{35}S]Methionine (>600 Ci/mmol) was pur-

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chased from the Radiochemical Centre, Amersham. Dexamethasone was from Sigma, Munich, and from Merck, Darmstadt, RPMI 1640 medium was from Serva, Heidelberg. Lipopolysaccharide derived from *Salmonella minnesota* R595 was a generous gift from Dr C. Galanos, Max-Planck-Institut für Immunbiologie, Freiburg.

Male Wistar rats were hypophysectomized according to the method originally developed by Fedotov and Bagramjan as described in [28]. Animals which showed total atrophy of their testes and no increase in body weight were used. The hypophysectomized animals were kindly supplied by Thomae, Biberach, FRG.

Suspensions of rat hepatocytes were prepared as in [29]. The hepatocyte primary cultures consisted of >97% hepatocytes. Before each experiment the cultures were routinely controlled by microscopic examination. Labeling of hepatocytes was carried out as in [30–32]. The preparation of antisera against rat α_2M and α_1AGP and the immunoprecipitations have been described [33,34]. For the quantification of the radioactivity incorporated into α_2M, the respective bands identified by fluorography [35] were cut from the SDS–PAGE gels [36], solubilized with protosol/water (9:1, v/v) at 45°C overnight and counted in a liquid scintillation spectrometer.

Kupffer cells were isolated by pronase perfusion of livers removed aseptically from female Wistar rats (160–180 g) and maintained in primary cultures as in [37]. Changes of the medium and determination of the viability and purity of the cells were performed as in [38]. 48-h primary cultures (1×10^6 cells/dish) of Kupffer cells were washed with Eagle's medium supplemented with 50 mM Hepes buffer, pH 7.4. The cells were then incubated in RPMI 1640 medium (3 ml/dish), supplemented with 10% newborn calf serum and maintained in culture for an additional 24 h with or without LPS (30 μg/ml). Thereafter the Kupffer cell media were removed from the culture dishes, filtered under sterile conditions through a 0.2 μm filter, dialyzed against 10 mM Hepes, pH 7.4, 0.15 M NaCl overnight and used for the stimulation of hepatocytes.

3. RESULTS AND DISCUSSION

Adult rats were injected with dexamethasone, turpentine or dexamethasone plus turpentine. The responses were studied and compared to those of hypophysectomized animals. Unlike turpentine injection into normal rats (fig.1A II), the administration of dexamethasone did not lead to any detectable increase in serum α_2M concentrations (fig.1A I). After the simultaneous application of dexamethasone and turpentine the maximum of the α_2M concentration in serum was reached several hours earlier than after turpentine injection. If turpentine was injected into hypophysectomized animals where nearly no endogenous glucocorticoids were present, no increase in serum α_2M levels could be observed (fig.1B II). Because dexamethasone plus turpentine injection into hypophysectomized rats (fig.1B III) resulted in an α_2M response similar to that in control animals after turpentine, a permissive action of glucocorticoids in the acute phase protein response is evident.

When α_1AGP concentrations were determined in the same sera, glucocorticoids were also found to exert a permissive effect (fig.1C,D). If no glucocorticoids were present, turpentine-induced inflammation did not result in a comparable effect on α_1AGP concentrations (fig.1D II) as observed in normal rats during inflammation (fig.1C II). Small increases in α_1AGP levels were observed in serum of turpentine-treated, hypophysectomized rats. The injection of dexamethasone alone had a slight stimulatory effect on the α_1AGP serum levels in normal as well as in hypophysectomized rats (fig.1C I and D I).

The finding that compared to the induction observed after turpentine injection, dexamethasone administered in vivo led only to slight increases in plasma levels of α_1AGP and to no detectable increase in α_2M was in accordance with low in vitro translational mRNA activities for both proteins, amounting only to about 10% of those mRNA levels after turpentine induction (not shown).

In contrast to the in vivo experiments we have recently described, dexamethasone alone is capable of stimulating α_2M synthesis in rat hepatocyte primary cultures [17]. Thus, hepatocyte primary cultures probably contain either a second factor required for the induction of α_2M synthesis or a repressor has been removed during the preparation of hepatocytes. We screened for a factor which

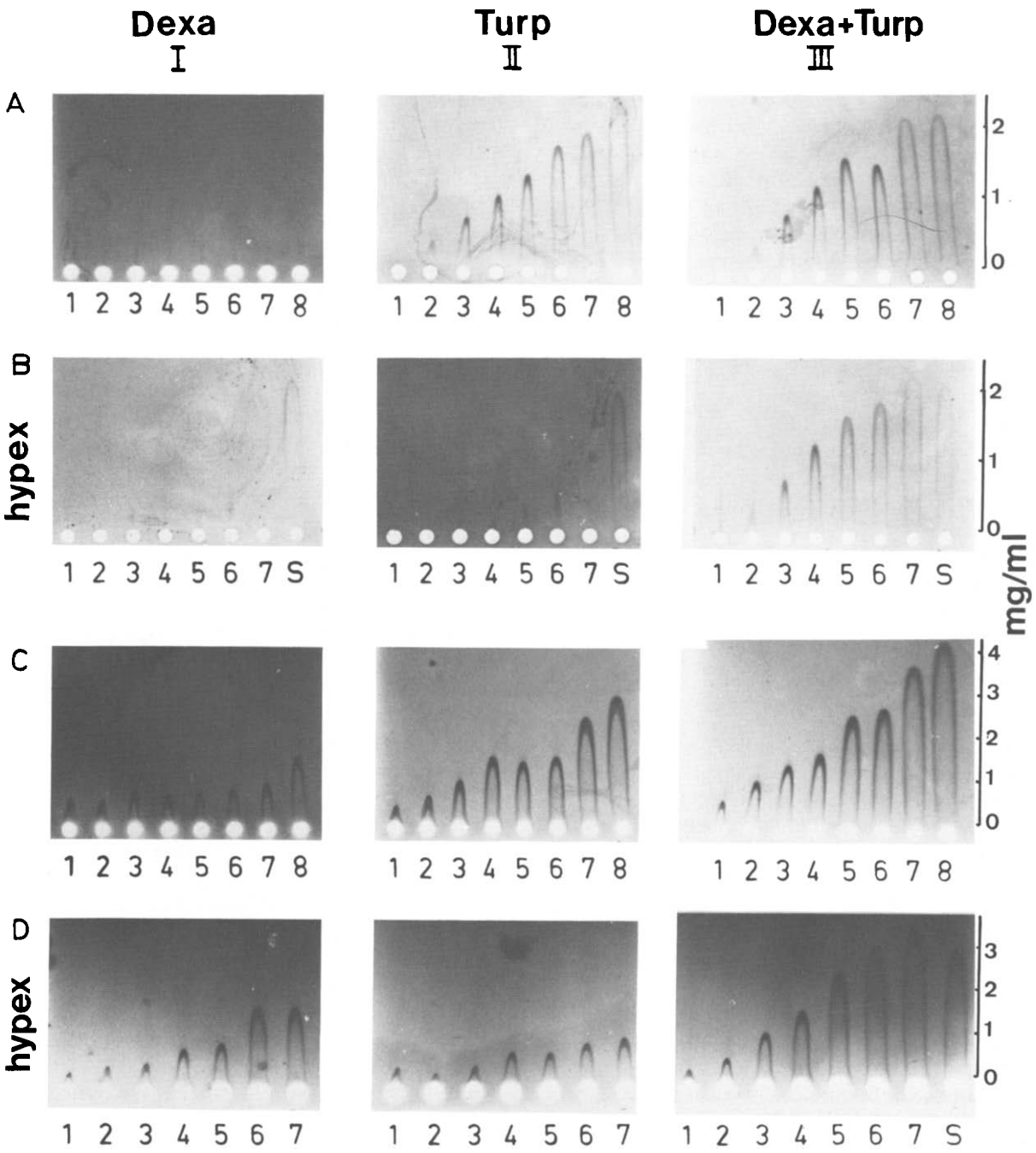

Fig.1. Determination of rat serum α_2-macroglobulin and α_1-acid glycoprotein by quantitative rocket immunoelectrophoresis. Dexamethasone (4 mg/kg body wt, injected i.p.), turpentine (5 ml/kg body wt, administered i.m.) or dexamethasone plus turpentine were given to normal (A,C) and hypophysectomized (B,D) male Wistar rats. At 0 h (1), 6 h (2), 9 h (3), 12 h (4), 15 h (5), 18 h (6), 24 h (7) and 40 h (8) after dexamethasone (I), turpentine (II) and dexamethasone plus turpentine (III) injection, serum was obtained and diluted 16-fold with water. 5-μl aliquots were applied to a 10% agarose gel containing about 10% α_2M- or α_1AGP antiserum. The immunoelectrophoresis was performed at 2 V/cm essentially as in [39]. The rocket on the right of the gels with sera from hypophysectomized animals represents serum from normal rats 24 h after turpentine treatment as a standard (S).

could further stimulate α_2M synthesis in rat hepatocyte primary cultures, being aware of the fact that only a small range of stimulation could now be expected.

Macrophage-derived factors have been described to elicit the acute phase response [18–27]. Since Kupffer cells represent the majority in the macrophage-phagocyte system and are in close proximity to hepatocytes, we considered Kupffer cells as primary candidates for the synthesis of a

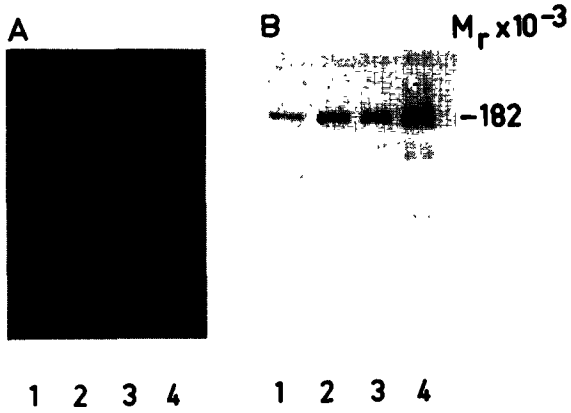

Fig.2. Stimulation of α_2-macroglobulin synthesis in rat hepatocytes by supernatants of rat Kupffer cells. Hepatocyte primary cultures (3.9×10^6 cells per dish) were incubated for 10 h in the presence of 10^{-9} M dexamethasone in a total volume of 3 ml containing increasing amounts of media of LPS-activated rat Kupffer cells as described in section 2. The hepatocyte medium consisted of 1 ml Waymouth medium and 2 ml RPMI 1640 medium. The latter was also used for the incubation of rat Kupffer cells, thereafter designated as supernatant. RPMI medium in the hepatocyte incubation medium was replaced by media of rat Kupffer cells: no addition (lane 1), 0.2 ml (lane 2), 0.5 ml (lane 3) and 2 ml (lane 4). The media of all dishes including the control contained LPS at a final concentration of 20 μg/ml. Incubation was carried out at 37°C for 10 h, the media were removed and replaced by 3 ml Waymouth medium without methionine. 25 μCi [^{35}S]methionine were added to each dish. After a labeling period of 90 min cells (A) and media (B) were separated and α_2M was immunoprecipitated. Comparable amounts of total trichloroacetic acid-precipitable radioactivity were used for the immunoprecipitation (7×10^6 cpm in the case of cells and 1×10^6 cpm in the case of media). Two individual dishes were used for each experiment and pooled before immunoprecipitation.

Table 1

Effect of media from rat Kupffer cell cultures on the synthesis of α_2-macroglobulin in rat hepatocyte primary cultures

Kupffer cell medium (ml)	Immunoprecipitated α_2M from	
	Cells (%) ($\times 10^2$)[a]	Medium (%) ($\times 10^2$)[b]
Control	2.5	4.8
0.2	4.6	8.2
0.5	4.8	8.3
2.0	8.3	13.7

The radioactively labeled α_2M bands of fig.2 were cut from the gels and the radioactivity was determined. [a] The data are percentages of total trichloroacetic acid-precipitable radioactivity in hepatocytes and [b] hepatocyte medium

hepatocyte stimulating factor. We studied the effect of dialyzed media from LPS-stimulated rat Kupffer cell cultures on rat hepatocytes that were kept in 10^{-9} M dexamethasone. As shown in fig.2 increasing amounts of Kupffer cell supernatants added to hepatocytes led to a dose-dependent increase in de novo α_2M synthesis in cells (A) and medium (B). The weak bands in the autoradiogram above the 182-kDa α_2M band in fig.2A are due to artifacts related to immunoprecipitation. When dexamethasone was omitted, no stimulation of α_2M synthesis was observed (not shown). For further quantification the α_2M bands of fig.2 were excised from the gels and their radioactivity was determined. The data in table 1 show a 3.4- and 2.9-fold increase in de novo α_2M synthesis in hepatocytes and hepatocyte media, respectively.

It is of interest to note that α_2M synthesis could be induced to the same extent by a supernatant from LPS-stimulated as well as unstimulated Kupffer cells or human monocytes [40]. This suggests that interleukin 1 can be excluded as the hepatocyte stimulating factor, since the supernatants of LPS-stimulated and non-stimulated human monocytes differ about 10-fold in respect to their interleukin 1 activities.

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