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Glucocorticoids and catecholamines as mediators of acute-phase proteins, especially rat α-macrofoetoprotein

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Adrenal hormones were studied as possible triggering substances of the synthesis of acute-phase reactants in rats. α-Macrofoetoprotein, which rises sharply in concentration during inflammation, was used to monitor the acute-phase reaction. In normal rats glucocorticoids and catecholamines induce α-macrofoetoprotein synthesis; glucocorticoids only increase α-macrofoetoprotein to moderate levels in plasma, but catecholamines enhance α-macrofoetoprotein synthesis to very high levels, comparable with those observed in the post-injury phase. However, catecholamines *in vivo* also activate the adrenal cortex, suggesting a synergistic effect of both kinds of adrenal hormones. Our study showed that in adrenalectomized rats, the effect of catecholamines on α-macrofoetoprotein synthesis is greatly diminished, whereas the moderate effect of glucocorticoids remains. Combination of glucocorticoids and catecholamines induces extremely high α-macrofoetoprotein levels in both adrenalectomized and normal rats. With crossed immunoelectrophoresis it was shown that other acute-phase reactants, such as haptoglobin and α_1-major acute-phase protein, are affected differently by the hormones. Contrary to glucocorticoids, catecholamines give a pattern comparable with that found after surgical injury.

Acute-phase proteins (or acute-phase reactants) are of importance in modulating acute inflammatory reactions and repair processes (Bonta, 1978; van Gool *et al.*, 1978). The system regulating the synthesis of these acute-phase reactants is largely unknown; therefore we analysed cause and pattern of acute-phase reactant response in the rat. The rat has a very typical acute-phase reactant, α-macrofoetoprotein, showing very low plasma levels in normal animals (10–100 μg/ml), but rising sharply after injury to 3000–8000 μg/ml within 24h (van Gool *et al.*, 1977). We chose this protein to study the triggering mechanism of the acute-phase reaction. The protein is a strong inhibitor of inflammatory mediators such as histamine, bradykinin, serotonin and prostaglandin E_2. It also inhibits polymorphonuclear chemotaxis (van Gool *et al.*, 1982) and more complicated inflammatory reactions such as carrageenan oedema and galactosamine hepatitis (van Gool *et al.*, 1978).

The protein is produced by the liver (Thompson *et al.*, 1976) and *in vivo* production starts within 4h after injury (van Gool & Ladiges, 1969). The triggering mechanism therefore operates very rapidly, indicating hormonal action, especially of adrenal hormones. For α-macrofoetoprotein and other acute-phase reactants the importance of glucocorticoids as permissive substances has already been described. Additional factors from leucocytes (interleukin I) and/or hormones such as insulin and thyroxin, and the degree of tissue necrosis itself, are also considered to be of importance (Baumann *et al.*, 1983; Jeejeebhoy *et al.*, 1977; Thompson *et al.*, 1976).

We re-investigated the problem but restricted our studies to glucocorticoids, corticotropin, adrenaline and noradrenaline as mediators, and to α-macrofoetoprotein as a typical acute-phase reactant of the rat. We also paid some attention to haptoglobin and α_1-major acute-phase protein (Schreiber *et al.*, 1982; Urban *et al.*, 1979).

The questions we tried to answer were as follows. Is it possible to induce without tissue injury an acute-phase response of α-macrofoetoprotein using corticosterone, the natural rat glucocorticoid? If so, is this response comparable with that observed after surgical injury? And if not, what other triggering mechanism exists? Here we

especially investigated the role of catecholamines, as it is known that adrenalectomy strongly suppresses the acute phase response (Heim & Ellenson, 1965).

Materials and methods

Chemicals

Dexamethasone sodium phosphate (Oradexon; 5mg/ml) and cortisone acetate (Adreson; 25mg/ml) were from Organon, Oss, The Netherlands. Hydrocortisone sodium succinate (Solu-Cortef; 50 mg/ml) was from Upjohn, Puurs, Belgium, and corticosterone from Fluka AG, Buchs, Switzerland. Adrenaline bitartrate and noradrenaline bitartrate were supplied by Calbiochem, San Diego, CA, U.S.A. Corticotropin (Synacthen; 1mg/ml) was obtained from Ciba-Geigy, Groot-Bijgaarden, Belgium. Sodium pentobarbitone (Nembutal; 60mg/ml) was from Ceva, Neuilly-sur-Seine, France and narcotic diethyl ether from Hoechst AG, Frankfurt am Main, Germany. Agarose M was supplied by LKB, Bromma, Sweden. All other chemicals were AR grade, where available.

Experimental procedure

Male Wistar rats (SPF-free), weight 200–250g, were obtained from TNO, Zeist, The Netherlands. Adrenalectomy was performed bilaterally under pentobarbital anaesthesia using a paravertebral dorsal approach. After adrenalectomy the animals received a 0.14M-NaCl solution instead of normal drinking water, were maintained on a 12h light/12h dark cycle, and were allowed to eat *ad libitum*.

Glucocorticoids were given orally, corticotropin was administered intramuscularly, and adrenaline and noradrenaline were injected either subcutaneously or intraperitoneally. Blood sampling was performed by puncture of the orbital plexus.

The experiments were performed with pentobarbital anaesthesia or under ether anaesthesia.

Corticosteroid determination

Corticosteroids were determined by an improved fluorimetric method (Silber *et al.*, 1958), with slight modifications involving methylene chloride for the extraction of the plasma. The fluorescence reagent consisted of 3 vol. of ethanol to which was added, with sufficient cooling in an ice bath, 7 vol. of concentrated H_2SO_4.

Preparation of antiserum

Antiserum to rat post-injury serum, obtained 24h after intraperitoneal injection of 2ml of a 40% (w/v) $BaSO_4$ suspension in saline, was prepared in a rabbit (van Gool *et al.*, 1978).

Three injections of 0.5 ml of rat serum in 0.5ml of Freund's adjuvant were given intramuscularly at monthly intervals. The first and second injections were given with complete, and the third with incomplete, Freund's adjuvant. At 4 weeks after the last injection the rabbit received a booster injection of 0.5ml of serum only. This booster injection was repeated 2 weeks later. Thereafter the animal was bled twice weekly, and further booster injections were given when necessary. After obtaining a sufficient amount of antiserum, the batches were pooled. The same antiserum pool was used throughout.

Immunological techniques

Crossed immunoelectrophoresis of rat serum was performed in an LKB Multiphor apparatus according to the method of Clarke & Freeman (1968), using slight modifications. Agarose M, 1%, dissolved in electrophoretic buffer [Tris/barbiturate (pH 8.6, $I = 0.02$) containing 0.45mM-calcium lactate and 2mM-NaN_3] was poured on a glass plate (84mm × 94mm). First-dimension electrophoresis was performed with 1μl of undiluted serum for 1.5h at 10V/cm and 11°C. The separated proteins were then run at right angles to the first electrophoretic direction into a gel containing 0.3ml of the appropriate antiserum. Electrophoretic separation and immunoprecipitation in the second direction was continued for 22h at 2V/cm, also at 11°C. After repeated washings the plates were dried and stained for proteins with Coomassie Brilliant Blue. When the patterns of acute-phase serum and normal serum are compared, apart from α-macrofoetoprotein four clearly defined peaks (1–4) representing other acute-phase reactants are detectable. For peaks 1–3 we measured the peak height, whereas for peak 4 the area of the total peak was determined. α-Macrofoetoprotein, though visible, was measured separately by the Mancini method with monospecific rabbit antiserum (van Gool *et al.*, 1978).

Statistics

Data concerning α-macrofoetoprotein and corticosteroids were analysed statistically by means of Student's *t*-test (*Documenta Geigy*, 1977).

Results

Effect of surgical injury and adrenalectomy on α-macrofoetoprotein response

The basal serum concentration of α-macrofoetoprotein in uninjured rats is rather variable (10–100μg/ml). Laparotomy (under sterile conditions and pentobarbital anaesthesia) always provoked a strong α-macrofoetoprotein reaction (up to 3000–4000μg/ml).

It was shown some years ago that adrenalectomy diminishes this response (Heim & Ellenson, 1965), but does not abolish it completely. In these experiments the effect of the surgical procedures necessary to perform the adrenalectomy must be considered; therefore we measured α-macrofoetoprotein in serum just before operation and 24h later. The results are summarized in Table 1.

From these results we conclude that anaesthesia alone (but with blood sampling twice) induces a small but significant increase of α-macrofoetoprotein ($P < 0.05$), whereas a gross injury such as laparotomy (with blood sampling twice) induces a brisk increase of plasma α-macrofoetoprotein ($P < 0.001$). The same procedure with simultaneous adrenalectomy strongly diminishes this response but does not abolish it completely ($P < 0.02$).

Basal values of α-macrofoetoprotein and plasma corticosteroids

We studied in normal rats the relation between plasma corticosteroids and the α-macrofoetoprotein level. The mean plasma concentration of α-macrofoetoprotein was $53 \pm 6 \mu g/ml$ and the mean concentration of corticosteroids was $129 \pm 10 ng/ml$ ($n = 16$). No relationship was found ($r = 0.15$).

Ether anaesthesia under the same conditions provoked higher corticosteroid levels (292 ± 30 ng/ml, $n = 8$), also without significant relation to the α-macrofoetoprotein level measured 24h later.

Repeated blood sampling (three or four times) during 3h of pentobarbital anaesthesia resulted in higher corticosteroid levels (350–450 ng/ml). Apparently some adrenal cortical stimulation occurs during these circumstances, possibly inducing a slight but significant rise of α-macrofoetoprotein after 24h (see Table 1).

Effect of surgical injury (laparotomy) on plasma corticosteroids and α-macrofoetoprotein synthesis

Under pentobarbital anaesthesia a sterile laparotomy (length of incision 3cm) was performed in six healthy rats. Blood sampling was performed at 5 and 35 min (both in the same anaesthesia period) and 24h later. The results are presented in Table 2. This Table shows that laparotomy induces high but variable corticosteroid levels, declining after 24 h to normal values. At that time the α-macrofoetoprotein level is strongly increased.

Effect of glucocorticoids on plasma α-macrofoetoprotein in intact animals

Corticosteroid levels were measured 1, 2 and 3h after administration by stomach tube of different doses of corticosterone (Fig. 1). We used the oral route to avoid peripheral tissue lesions by subcutaneous or intramuscular injections. In all experiments oesophagus and stomach were studied, but no lesions were found. Our data are restricted to one determination per animal, avoiding the effect of repeated blood sampling on the corticosteroid level.

Fig. 1 indicates that corticosterone induces, in a dose-dependent manner, high corticosteroid levels after 1h, gradually declining during the next few hours. The effects of oral administration of different kinds of glucocorticoids on α-macrofoetoprotein levels are summarized in Table 3. This Table shows that dexamethasone is more effective than cortisone, cortisol or corticosterone. To obtain a level of $1000 \mu g$ of α-macrofoetoprotein/ml, sufficient to inhibit inflammatory reactions (van Gool *et al.*, 1978), 0.35mg of dexamethasone/kg body wt. is needed. Cortisol and corticosterone require about 40mg/kg and 30mg/kg, respectively. Cortisone is especially ineffective. Although dexamethasone has a strong action, it cannot be the natural trigger of α-macrofoetoprotein synthesis. Corticosterone has a rather weak action, but this does not exclude a permissive role of this glucocorticoid. Because of these consider-

Table 1. *α-Macrofoetoprotein concentration in rat serum after different kinds of injury*

Values are means ± S.E.M. for the numbers of rats given in parentheses, just before the operation ($t = 0$) and 24h afterwards. *P* values are: *a* versus *b*, <0.05; *c* versus *d*, <0.001; *e* versus *f*, <0.01; *b* versus *d*, <0.001; *d* versus *f*, <0.001; *b* versus *f*, <0.02.

Kind of injury	α-Macrofoetoprotein (μg/ml) at time: 0	24h
Pentobarbital anaesthesia	68 ± 10 (10)[a]	106 ± 12 (10)[b]
Pentobarbital anaesthesia plus laparotomy	53 ± 4 (12)[c]	3423 ± 474 (12)[d]
Pentobarbital anaesthesia plus bilateral adrenalectomy	75 ± 6 (10)[e]	231 ± 26 (10)[f]

Table 2. *Plasma corticosteroids and α-macrofoetoprotein at different times after laparotomy*

Values are means ± S.E.M. for the numbers of rats given in parentheses.

Time after laparotomy	Corticosteroids (ng/ml)	α-Macrofoetoprotein (μg/ml)
5 min	232 ± 30 (6)	55 ± 7 (6)
35 min	795 ± 87 (6)	–
24 h	167 ± 21 (6)	2891 ± 301 (6)

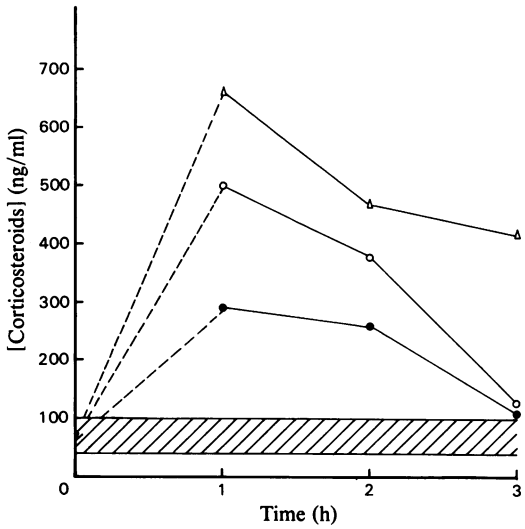

Fig. 1. *Time course of the absorption of orally administered corticosterone*
To avoid the effect of repeated blood sampling, data are restricted to one determination per animal. Dosages: ●, 5mg/kg body wt.; ○, 10mg/kg body wt.; △, 20mg/kg body wt. The hatched area represents the plasma corticosteroid level found in normal rats.

ations we also studied the effects of adrenaline and noradrenaline on α-macrofoetoprotein synthesis.

Effect of catecholamines on α-macrofoetoprotein synthesis in the intact rat

In pilot studies using adrenaline and noradrenaline, different dosages and time schedules were studied. No anaesthesia was used except for blood sampling at the start of the experiment and 24h later (pentobarbital). Subcutaneous administration sometimes provoked a slight local oedema. To avoid this we also used the intraperitoneal route, but no differences in α-macrofoetoprotein synthesis were observed (Table 4). After intraperitoneal administration we found no lesions in the abdominal cavity.

Blood pressure after intraperitoneal administration of 0.2mg of adrenaline or noradrenaline showed only a temporary (5–10min) rise from 80 to 140mmHg. The results are summarized in Table 4(*a*); our conclusion is that catecholamines (adrenaline and noradrenaline) are, dependent upon dose, very strong stimulants of α-macrofoetoprotein production. One injection of only 0.5mg of adrenaline induces high α-macrofoetoprotein levels. The effect of noradrenaline does not seem significantly less compared with that of adrenaline.

Table 3. *α-Macrofoetoprotein levels in rat serum 24h after a single oral dosage of glucocorticoids*
Values are means ± S.E.M. for the numbers of rats given in parentheses. The mean value of α-macrofoetoprotein of control rats was 53 ± 4 μg/ml ($n = 12$).

Glucocorticoid	Dosage (mg/kg body wt.)	α-Macrofoetoprotein (μg/ml)
Dexamethasone	0.06	387 ± 75 (4)
	0.12	483 ± 142 (4)
	0.25	402 ± 170 (4)
	0.5	1700 ± 172 (4)
	1.0	1501 ± 360 (4)
	2.0	2132 ± 130 (4)
Cortisone	2.5	188 ± 59 (4)
	5.0	140 ± 16 (4)
	10.0	151 ± 53 (7)
	20.0	228 ± 85 (4)
Cortisol	12.5	639 (2)
	45.0	1610 ± 295 (5)
Corticosterone	2.5	218 ± 202 (3)
	5.0	506 ± 70 (8)
	10.0	916 ± 187 (6)
	20.0	765 ± 195 (6)
	40.0	1205 ± 643 (4)

Effect of adrenaline on plasma corticosteroids in relation to α-macrofoetoprotein

As it is an old dispute (Ganong, 1963) as to whether catecholamines in the rat stimulate the pituitary–adrenal cortex system, we measured this response 1h after adrenaline administration, as well as measuring α-macrofoetoprotein 24h later. The data are summarized in Table 4(*b*), indicating that one single injection of adrenaline suffices to increase plasma corticosteroids. Low and high doses have nearly the same effect on adrenal cortex response, but their effect on α-macrofoetoprotein synthesis differs.

Effect of corticotropin on plasma corticosteroids and α-macrofoetoprotein

To investigate whether long-acting corticotropin has a more pronounced effect, depot-corticotropin, 25μg and 100μg, was administered intramuscularly under pentobarbital anaesthesia ($n = 6$). Plasma corticosteroids were measured at 0, 20, 30, 60 and 120min and α-macrofoetoprotein 24h later. The α-macrofoetoprotein response was relatively small and varied from 194 to 980μg/ml. Corticosteroid levels rose to 550ng/ml maximally, being a response similar to that obtained with 10–20mg of corticosterone orally (see Table 3). Here also we conclude that the α-macrofoetoprotein response obtained after surgical injury cannot be

Table 4. *α-Macrofoetoprotein 24h after the first dosage of catecholamines, administered either subcutaneously or intraperitoneally as indicated*

When repeated doses were given the time schedule was 0, 2, 4 and 7h. Plasma corticosteroids were measured (ng/ml) 1h after a single dose of catecholamines. Values are means ± S.E.M.; the numbers of rats are given in parentheses. Mean value of α-macrofoetoprotein of control rats given saline intraperitoneally was 46 ± 9 μg/ml) ($n = 5$).

	Catecholamine	Dosage (mg)	Route	α-Macrofoetoprotein (μg/ml)	Corticosteroids (ng/ml)
(*a*)					
	Adrenaline	4 × 0.05	Subcutaneous	162 ± 17 (4)	–
		4 × 0.10	Subcutaneous	1570 ± 168 (4)	–
		4 × 0.20	Subcutaneous	4031 ± 710 (7)	–
		4 × 0.05	Intraperitoneal	317 ± 108 (4)	–
		4 × 0.20	Intraperitoneal	4346 ± 642 (4)	–
	Noradrenaline	4 × 0.05	Subcutaneous	154 ± 25 (3)	–
		4 × 0.10	Subcutaneous	988 ± 266 (3)	–
		4 × 0.20	Subcutaneous	2062 ± 130 (3)	–
(*b*)					
	Adrenaline	1 × 0.05	Subcutaneous	–	530 ± 24 (3)
		1 × 0.10	Subcutaneous	1363 ± 234 (3)	550 ± 10 (3)
		1 × 0.20	Subcutaneous	2794 ± 270 (5)	620 ± 39 (5)

explained fully by stimulation of the adrenal cortex.

Effect of glucocorticoids and adrenaline on α-macrofoetoprotein levels in adrenalectomized rats

The foregoing experiments strongly suggest that besides glucocorticoids, catecholamines are also stimulating factors. To study this relation in more detail, we used adrenalectomized rats, avoiding the cortical response to adrenaline shown in Table 4(*b*).

After adrenalectomy the animals were left for 8–10 days to recover. Control experiments showed that adrenalectomy was complete and that cortical stimulation had no effect at all on plasma corticosteroids, which remained very low (i.e. 53 ± 5 ng/ml, $n = 7$). Orally administered corticosterone was absorbed quickly, reaching plasma levels comparable with that of normal animals (i.e. 640 ± 80 ng/ml, $n = 7$) after 45 min (see Fig. 1).

The effect of glucocorticoids and catecholamines separately or in combination is shown in Table 5.

Table 5. *Effect of glucocorticoids or catecholamines administered separately or in combination on α-macrofoetoprotein levels after 24h in adrenalectomized rats*

Values are means ± S.E.M. for the number of rats given in parentheses. Doses of glucocorticoids are given as mg/kg body wt.

Stimulants	α-Macrofoetoprotein (μg/ml)
None	170 ± 12 (14)
Corticosterone (5 mg/kg)	1287 ± 315 (9)
Dexamethasone (2 mg/kg)	1550 ± 225 (8)
Adrenaline (4 × 0.2 mg subcutaneously)	533 ± 212 (6)
Corticosterone (5 mg/kg) + adrenaline (4 × 0.2 mg subcutaneously)	2717 ± 363 (10)
Dexamethasone (2 mg/kg) + adrenaline (4 × 0.2 mg subcutaneously)	7000 ± 900 (5)
Laparotomy	231 ± 26 (10)
Laparotomy + corticosterone (5 mg/kg)	3217 ± 603 (7)

Dexamethasone induces α-macrofoetoprotein synthesis to moderate levels, comparable with those in intact animals (compare Table 3), whereas the response to corticosterone in adrenalectomized rats is somewhat higher, a phenomenon also known for fibrinogen synthesis triggered by cortisol (Crane & Miller, 1977). Adrenalectomy suppresses the effect of adrenaline. However, when glucocorticoids and adrenaline are given together a strong synergistic effect is observed (Table 5). This effect is far more than that expected by addition. This is also the case in normal rats, in which the combination of dexamethasone and adrenaline gives extremely high levels of α-macrofoetoprotein (mean 7000 μg/ml). Adrenalectomy also inhibits the response of α-macrofoetoprotein to surgical injury, where it remains low (Table 5). However, laparotomy in adrenalectomized rats supplemented with corticosterone induces α-macrofoetoprotein levels similar to those in intact animals (Tables 1 and 5).

All these results suggest that, besides glucocorticoids, catecholamines are of importance to the acute-phase reaction of α-macrofoetoprotein.

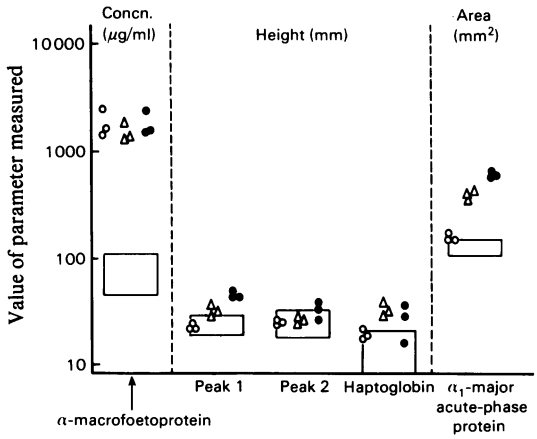

Fig. 2. *Course of five different acute-phase reactants of the rat, 24 h after different situations triggering an acute-phase reaction*

Rats were induced to an acute-phase reaction, 24 h previously, by: ○, 24 mg of corticosterone/kg body wt., orally; △, 0.2 mg of adrenaline, subcutaneously; ●, a surgical injury. Peaks 1 and 2 and haptoglobin were determined by the height of the peaks in the crossed immunoelectrophoresis, whereas α_1-major acute-phase protein of the rat was calculated by measuring the area of the total peak. α-Macrofoetoprotein was measured by a Mancini technique. The open rectangles represent the range for normal rats.

Effect of injury, adrenaline and corticosteroids on other acute-phase reactants of the rat

With crossed immunoelectrophoresis (see the Materials and methods section), at least four other acute-phase protein peaks are clearly distinguishable: haptoglobin, α_1-major acute-phase protein (Urban *et al.*, 1979; Schreiber *et al.*, 1982) and peaks 1 and 2, not yet identified. A distinct difference exists in the reaction of peak 1, haptoglobin and α-major acute-phase protein to adrenaline and corticosterone. These differences are represented graphically in Fig. 2. For this study we selected acute-phase sera with the same level of α-macrofoetoprotein (about $1750 \pm 150\,\mu g/ml$, $n = 9$) in order to compare acute-phase reactant patterns in different situations with α-macrofoetoprotein as a standard. Although more experiments have to be performed, Fig. 2 shows that surgical injury and adrenaline induce the same pattern of acute-phase reactants (α-macrofoetoprotein, haptoglobin, α_1-major acute-phase protein, peaks 1 and 2), whereas corticosterone only induces α-macrofoetoprotein and to a much lesser degree haptoglobin and α_1-major acute-phase protein. These results show that the triggering factors for various acute-phase reactants are different, at least quantitatively. Adrenaline induces a much broader spectrum than do glucocorticoids.

Discussion

As stated in the introduction, much uncertainty exists about the triggering mechanism inducing synthesis of acute-phase reactants. Jeejeebhoy *et al.* (1977) showed that synthesis of α_1-acid glycoprotein is promoted by glucocorticoids, insulin and thyroxin. Shim & Hong (1981) found that prostaglandins raised plasma haptoglobin and caeruloplasmin. In the case of α-macrofoetoprotein, a foetal acute-phase reactant of the rat with anti-inflammatory properties, some experiments point to a factor released from the injured area (van Gool & Ladiges, 1969), possibly a leucocytic factor liberated from disintegrating leucocytes in the injured area (Thompson *et al.*, 1976). However, the administration of extracts and the like often induces stress reactions, and therefore adrenal reactivity has to be considered during experiments *in vivo*. Thus also in the case of α-macrofoetoprotein, glucocorticoids and catecholamines remain candidates as triggering factors. We found that, apart from surgical injury itself, anaesthesia and repeated blood sampling also induce a stress situation with higher corticosteroid levels, followed by slightly elevated α-macrofoetoprotein concentrations (see the Results section and Tables 1 and 2). In accordance with this, oral administration of glucocorticoids induces moderately raised α-macrofoetoprotein levels (see Table 3), but even with dexamethasone, having the strongest effect in this respect, α-macrofoetoprotein remains below levels observed after surgical trauma such as laparotomy (compare Tables 1 and 2 with Table 3). In particular corticosterone, the natural glucocorticoid of the rat, has a rather weak action, even in high dosage (Table 3).

Our conclusion is that the complete acute-phase reaction of α-macrofoetoprotein in an intact animal can only be partially explained by glucocorticoid action; other stimulatory factors must be present. As the injured rat shows a rapid rise of adrenaline, followed by noradrenaline (Egger *et al.*, 1982), we studied the effect on α-macrofoetoprotein *in vivo* of physiological doses of these catecholamines. High α-macrofoetoprotein levels and a dose-dependent relationship were observed (see Table 4*a*). No local tissue lesions were present in these experiments.

These observations suggest that, besides glucocorticoids, catecholamines can also trigger the acute-phase reaction of α-macrofoetoprotein. However, this effect could be an indirect one because, in the rat, catecholamines activate the

adrenal cortex (Ganong, 1963), as we could confirm (see Table 4*b*).

In adrenalectomized rats the acute-phase response after injury is strongly depressed (Table 1), but corticosterone maintains its (moderate) effect, whereas adrenaline does not (see Table 4). This indicates that the catecholamine effect needs cortical activity as a permissive action. The combination of adrenaline with dexamethasone or corticosterone in the adrenalectomized animal restores α-macrofoetoprotein production completely in a synergistic way, and less so as an additive effect. This is in accordance with other studies on experimental inflammation (Sendelbeck & Yates, 1970; Egger *et al.*, 1982). The same was found for fibrinogen (Crane & Miller, 1977). When surgical injury in adrenalectomized animals is combined with a fixed dose of corticosterone, high α-macrofoetoprotein levels are obtained, comparable with the situation in normal rats but higher than obtained with corticosterone alone (Table 2). Another argument for a separate action of glucocorticoids and catecholamines on the acute-phase reaction is the different effect they have on various kinds of acute-phase reactants. Glucocorticoids induce α-macrofoetoprotein but have no or a minor effect on three other acute-phase reactants, in contrast with adrenaline (Fig. 2), which induces a much broader spectrum.

Our results show that an acute-phase reaction with α-macrofoetoprotein as parameter can be achieved without tissue lesion and only by synergistic action of glucocorticoids and adrenaline. Indeed, in our laboratory, we presently induce α-macrofoetoprotein up to levels of 6000–10000 μg/ml with one single dose of 2mg of dexamethasone orally combined with 0.2mg of adrenaline subcutaneously.

A part of our results can be explained by the fact that glucocorticoids affect the transcription phase of α-macrofoetoprotein synthesis (Baumann *et al.*, 1983), but the mechanism by which catecholamines stimulate synthesis of acute-phase reactants is less obvious. In this connection polyamines have to be considered. Ornithine decarboxylase is strongly activated during injury, resulting in a rapid production of polyamines in peripheral tissues as well as in the liver (Maudsley, 1979). Polyamines do affect protein synthesis (Abraham *et al.*, 1979; Sidransky *et al.*, 1982), and catecholamines induce either directly (Bartolomé *et al.*, 1977; Womble & Russell, 1983) or indirectly (Weiner & Ganong, 1978) a very high production of polyamines. Experiments are needed to examine the role of polyamines in the acute-phase reaction induced by adrenaline and corticosterone (Cousin *et al.*, 1982).

Our study does not exclude the possibility that other acute-phase reactants need another triggering system, but up to now most studies neglect the effects of catecholamines during the acute-phase reaction. As α-macrofoetoprotein has a human analogue (Boers *et al.*, 1979) with anti-inflammatory properties, our observations can be of importance for a better understanding of inflammatory reactions in man during stress, adrenal insufficiency and steroid therapy.

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