Biochemical Pharmacology, Vol. 36, No. 18, pp. 3069–3073, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00
Pergamon Journals Ltd.

ACUTE PHASE RESPONSES OF PLASMA ANGIOTENSINOGEN AND T-KININOGEN IN RATS

HIROSHI OKAMOTO,*† AKIKO HATTA,* NORIO ITOH,* YUTAKA OHASHI,* KAZUKO ARAKAWA* and SHIGETADA NAKANISHI‡

*Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kobe-Gakuin University, Kobe 673, Japan; and ‡Institute for Immunology, Kyoto University, Faculty of Medicine, Kyoto 606, Japan

(*Received* 28 *November* 1986; *accepted* 18 *February* 1987)

Abstract—Acute phase responses of plasma angiotensinogen and kininogen were studied in rats. Plasma angiotensinogen levels increased about 3-fold during the first 8 hr, and returned to normal at 48 hr, following the induction of acute inflammation by lipopolysaccharide (LPS). Plasma kininogen reached maximum levels at 48 hr following LPS administration. In adrenalectomized rats, plasma angiotensinogen levels decreased significantly, and the administration of LPS did not elevate plasma angiotensinogen levels. In contrast, plasma kininogen levels were increased by adrenalectomy, as well as by sham-operation. Dexamethasone significantly increased plasma angiotensinogen levels in adrenalectomized rats as well as in normal rats, but aldosterone did not. Plasma kininogen levels of normal rats were not changed by the administration of dexamethasone or aldosterone. From these results, it was concluded that the acute phase response of plasma angiotensinogen is mediated by glucocorticoid, but that of plasma kininogen is not.

Angiotensinogen is a plasma protein synthesized and secreted by the liver. It is a substrate for the circulating enzyme, renin (EC 3.4.99.19), and is, thus, the precursor of angiotensin II, the final product of the renin–angiotensin cascade [1]. Kininogens, including high molecular weight and low molecular weight kininogens, are also plasma proteins synthesized and secreted by the liver, and they are known to be protein precursors of kinins, released upon limited proteolysis by kallikreins [2].

Recently, a third kininogen, T-kininogen, from which trypsin releases T-kinin (Ile-Ser-bradykinin), was discovered in rat plasma [3, 4]. T-kininogen has been found to be responsible for the elevated kininogen level in plasma of rats with inflammatory lesions [5]. Subsequently, it was demonstrated that the induction of acute inflammation causes an increase in T-prekininogen mRNA levels of rat liver, but not in other prekininogen mRNA levels [6].

Like the acute phase response of hepatic T-prekininogen mRNA, a significant increase in angiotensinogen mRNA of liver was reported in rats following the induction of acute inflammation [7], although it is still unclear whether the response of hepatic angiotensinogen mRNA to the inflammation actually led to the elevation of the plasma angiotensinogen level.

In the present study, plasma angiotensinogen and kininogen levels were determined in normal and adrenalectomized rats following the induction of acute inflammation by lipopolysaccharide (LPS). The results indicate that LPS administration produced a rapid and significant elevation of plasma angiotensinogen levels. The present study also demonstrates an important role of glucocorticoid in the acute phase response of plasma angiotensinogen, but not of plasma kininogen.

†Address correspondence to: Hiroshi Okamoto, Ph.D., Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kobe-Gakuin University, Ikawadani-cho, Nishi-ku, Kobe 673, Japan.

MATERIALS AND METHODS

Materials. The following materials were obtained from commercial sources: [1-Asp,5-Ile]angiotensin I, bradykinin, [1-Tyr]kallidin, leupeptin and pepstatin (Peptide Institute Inc., Osaka, Japan); Na[^{125}I] (New England Nuclear, Boston, MA, U.S.A.); LPS (*Staphylococuss typhosa* 0901, Difco, Detroit, MI, U.S.A.); dexamethasone (Nakarai Chemicals, Kyoto, Japan); *d*-aldosterone (Sigma Chemical Co., St. Louis, MO, U.S.A.); DE-52 (Whatman, Clifton, NJ, U.S.A.) and Sephadex G15 and aminohexyl-Sepharose 4B (Pharmacia Japan, Tokyo, Japan).

Preparation of rat kidney renin. Renin was partially purified from rat kidneys by DE-52 chromatography and pepstatin-aminohexyl-Sepharose affinity chromatography as described by Matoba *et al.* [8]. The specific activity was 514 μg angiotensin I formed·(mg protein)$^{-1}$·hr^{-1} at pH 6.5, using bilaterally nephrectomized rat plasma as the substrate.

Animals and drugs. Male Sprague–Dawley rats (150–180 g) were used. LPS was dissolved in saline. Dexamethasone and aldosterone were dissolved in ethanol and diluted twenty times with saline. These drugs were injected i.p. into rats at a volume of 0.2 ml/100 g body weight.

Experiments with normal rats. LPS (1 mg/kg), dexamethasone (free base, 0.25 mg/kg) or *d*-aldosterone (free base, 0.25 mg/kg) was injected i.p. into normal rats. Immediately before (0 hr) and different times after (8, 16, 24, 48 and 72 hr) the treatments, the tail-tip of the animal was cut with a razor, and 100 μl of blood was collected into an

EDTA-treated capillary tube. The tube was centrifuged at 2000 *g* for 15 min, and plasma was obtained at each time.

Experiments with adrenalectomized rats. Adrenalectomy was performed bilaterally in rats under sodium pentobarbital anesthesia (30 mg/kg, i.p.). Animals were given tap water to drink throughout the experiments. Plasma was collected as described above, and 5 days after the adrenalectomy LPS (1 mg/kg), dexamethasone (0.25 mg/kg) or aldosterone (0.25 mg/kg) was injected intraperitoneally. Following the treatments, plasma was collected at different times as described in the experiments with normal rats.

Assay of angiotensinogen. Angiotensinogen levels in plasma were assayed by measuring the amounts of angiotensin I generated with a sufficient amount of partially purified renin. Plasma was diluted fifty times with 50 mM sodium phosphate (pH 6.5) containing 5 mM EDTA, 3 mM *o*-phenanthroline and 10 μM leupeptin (buffer A), and 50 μl of the sample was mixed with 50 μl of renin preparation (1.8 μg protein in buffer A). Following the incubation at 37° for 90 min, 400 μl of distilled water was added, and the tube was heated in a boiling water bath for 10 min. The sample was subjected to angiotensin I radioimmunoassay. Plasma angiotensinogen levels are expressed as μg angiotensin I per ml. The amount of renin used was twice the amount required for a complete generation of angiotensin I from bilaterally nephrectomized rat plasma (2% plasma). No degradation of angiotensin I was observed in either diluted plasma with buffer A or renin preparation.

Angiotensin I radioimmunoassay. Anti-angiotensin I antiserum was prepared in rabbits as described by Goodfriend *et al.* [9]. [^{125}I]-Labelled [1-Asp,5-Ile]angiotensin I was prepared by the chloramine-T method [10] and purified on Sephadex G-15 chromatography. The reaction mixture (total volume of 300 μl) contained angiotensin I tracer, diluted antiserum (1:60,000) and standard angiotensin I or sample in 0.1 M Tris–HCl (pH 7.4) containing 3 mM EDTA, 0.2% gelatin and 0.02% sodium azide. Following the incubation at 4° for 24 hr, the separation of antibody-bound and free [^{125}I]angiotensin I was carried out by the polyethylene glycol method [11]. Antibody-bound tracer in precipitates was counted by an Aloka gamma counter.

Assay of plasma kininogen. Plasma total kininogen was assayed by measuring the amount of kinin liberated by an excess amount of trypsin as described previously [3, 4]. Liberated kinin was determined by radioimmunoassay using rabbit anti-bradykinin serum and [^{125}I]-labeled [1-Tyr]-kallidin as described previously [5]. Plasma kininogen levels were expressed as μg bradykinin equivalents per ml.

Assay of plasma corticosterone. Plasma corticosterone was assayed fluorometrically by the method described by Silber [12].

RESULTS

Plasma angiotensinogen and kininogen levels in normal rats following the injection of LPS, dexamethasone and aldosterone. Acute inflammation was induced by a single intraperitoneal injection of LPS (1 mg/kg) into normal rats; plasma levels of angiotensinogen and kininogen were determined at various times. As seen in Fig. 1a, plasma angiotensinogen increased 3-fold over normal levels at 8 hr after LPS injection. Such high levels of plasma angiotensinogen were maintained for up to 24 hr and returned to normal levels at 48 hr after LPS injection. Plasma kininogen levels were also increased by LPS injection (Fig. 1b). Up to 48 hr after the treatment, plasma kininogen linearly increased by about 10-fold over the normal levels and still maintained high levels at 72 hr.

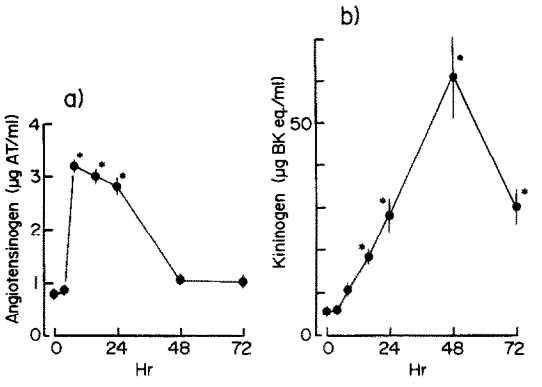

Fig. 1. Changes in the concentrations of plasma (a) angiotensinogen and (b) kininogen of normal rats following the injection of LPS. LPS (1 mg/kg) was injected i.p. into nine normal rats at 0 hr. Plasma was collected 0, 4, 8, 16, 24, 48 and 72 hr after the treatment, and the concentrations of angiotensinogen and kininogen were assayed. All animals survived the experimental period. Plasma levels of angiotensinogen and kininogens are expressed as μg angiotensin I per ml and μg bradykinin equivalents per ml respectively. Bars represent standard errors. Key: (*) significantly different from normal levels (0 hr) ($P < 0.001$).

As glucocorticoid has been known to stimulate hepatic angiotensinogen synthesis *in vivo* and *in vitro* [13, 14], the effects of dexamethasone on plasma levels of angiotensinogen and kininogen were examined. The plasma angiotensinogen level increased after an injection of dexamethasone (0.25 mg/kg)

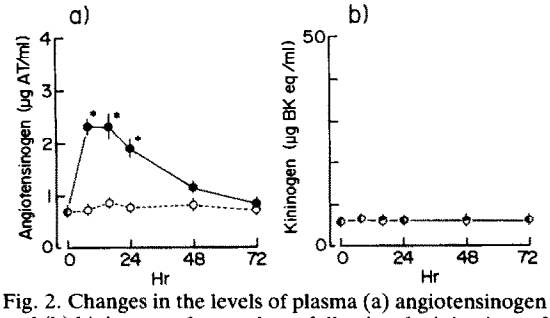

Fig. 2. Changes in the levels of plasma (a) angiotensinogen and (b) kininogen of normal rats following the injections of dexamethasone or aldosterone. Dexamethasone (0.25 mg/kg; —●—) or aldosterone (0.25 mg/kg; --○--) was injected i.p. into five normal rats at 0 hr. Other experimental conditions are described in the legend of Fig. 1. Key: (*) significantly different from normal levels (0 hr) ($P < 0.001$).

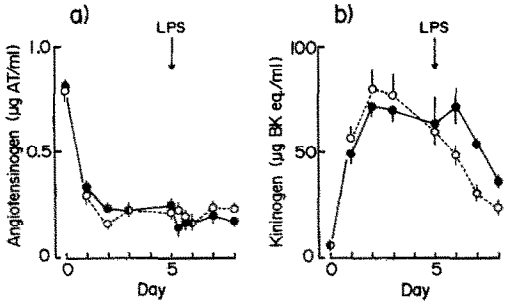

Fig. 3. Effects of LPS injection on the concentrations of plasma (a) angiotensinogen and (b) kininogen in adrenalectomized rats. Twelve rats were bilaterally adrenalectomized on day 0. On day 5, eight of them (–●–) were treated with LPS (1 mg/kg, i.p.) and another four (--○--) were treated with saline. Five of the eight animals receiving LPS died during the next 8 hr. All values for plasma angiotensinogen or kininogen levels after adrenalectomy were significantly different ($P < 0.001$) from each normal level (day 0) respectively. Other experimental conditions are described in the legend of Fig. 1.

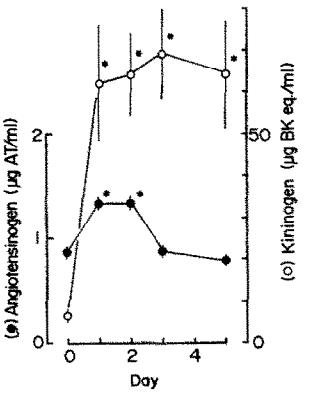

Fig. 5. Changes in the levels of plasma angiotensinogen and kininogen following sham-operation. On day 0, five rats were sham-operated, and, then, during the next 5 days plasma levels of angiotensinogen (–●–) and kininogen (–○–) were measured. Other experimental conditions are described in the legend of Fig. 1. Key: (*) significantly different from normal levels (day 0) ($P < 0.001$).

with a profile similar to that after LPS injection, but not after an injection of aldosterone (0.25 mg/kg) (Fig. 2a). Neither dexamethasone nor aldosterone injection caused any changes in plasma kininogen levels (Fig. 2b).

Plasma angiotensinogen and kininogen levels in adrenalectomized rats following the injection of LPS, dexamethasone and aldosterone. To clarify a possible role of steroids in the elevations of these plasma proteins in response to acute inflammation, bilaterally adrenalectomized rats were examined. As shown in Fig. 3a, plasma angiotensinogen levels exhibited a significant decrease after adrenalectomy. During the 8 hr following the injection of LPS, 5 days after the operation, five of eight animals died. Plasma angiotensinogen levels of the remaining three rats did not show any changes after the injection of LPS. In contrast to angiotensinogen, plasma kininogen levels were significantly elevated following adrenalectomy (Fig. 3a). Five days after the operation, LPS injection was followed by a slight elevation of kininogen over these high levels, though the response was very small in comparison to that by normal rats.

On the other hand, plasma angiotensinogen levels were significantly elevated after injection of dexamethasone but not after aldosterone injection (Fig. 4), like the responses of normal rats.

Plasma angiotensinogen and kininogen levels in sham-operated rats. To clarify whether the effects of adrenalectomy on plasma angiotensinogen and kininogen were due solely to tissue injury, plasma levels of these proteins were determined in rats following sham-operation. As shown in Fig. 5, plasma kininogen levels had increased by about 15-fold over the normal levels 3 days after the operation. In contrast to the adrenalectomy, the sham-operation itself caused an elevation of plasma angiotensinogen.

Plasma corticosterone levels of normal rats following injection of LPS. Changes in plasma corticosterone levels were determined following the injection of LPS. As shown in Fig. 6, plasma corticosterone levels significantly increased 8 hr, and attained maximum levels 16 hr, after the injection.

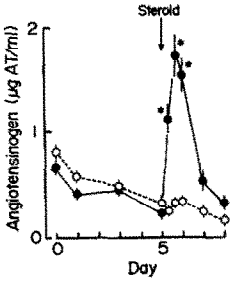

Fig. 4. Effect of injection of dexamethasone or aldosterone on the levels of plasma angiotensinogen in adrenalectomized rats. Twelve rats were adrenalectomized on day 0. On day 5, six (–●–) were treated with dexamethasone (0.25 mg/kg, i.p.), and the other six (--○--) were treated with aldosterone (0.25 mg/kg, i.p.). Other experimental conditions are described in the legend of Fig. 1. Key: (*) significantly different from day 0 value ($P < 0.001$).

DISCUSSION

A recent report of Kageyama *et al.* [7] demonstrated that acute inflammation induced by the injection of *Escherichia coli* LPS causes a rapid increase of angiotensinogen mRNA levels in rat liver. They observed that the level of angiotensinogen mRNA reached a maximum at 5–8 hr and returned to normal levels 24 hr after the injection of LPS. Coincidently with the response of hepatic mRNA, plasma angiotensinogen levels increased rapidly following an injection of LPS (Fig. 1). Thus, it is reasonable to conclude that the induction of hepatic angiotensinogen mRNA is responsible for elevation of plasma angiotensinogen in response to acute inflammation.

Kininogen, which responds to acute inflammation

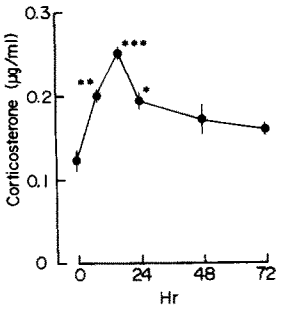

Fig. 6. Changes in the level of plasma corticosterone of normal rats following LPS injection. At 0 hr, LPS (1 mg/kg) was injected i.p. into normal rats. At each time point after LPS injection, four rats were killed and plasma corticosterone levels were determined. Other experimental conditions are described in the legend of Fig. 1. Key: significantly different from normal levels (0 hr): (*) $P < 0.05$, (**) $P < 0.01$, and (***) $P < 0.001$.

in rat, has been identified recently to be T-kininogen [5], a protein precursor of T-kinin (Ile-Ser-bradykinin). More than 90% of the kinins liberated by trypsin treatment of the plasma of rats receiving LPS consisted of T-kinin (data not shown), indicating that the increased kininogen shown in the present study is T-kininogen. As already reported by Kageyama *et al.* [6], the hepatic levels of T-prekininogen mRNA increase progressively in rats following induction of acute inflammation. Therefore, like angiotensinogen, the elevation of plasma kininogen level in response to acute inflammation also appears to be due to induction of hepatic T-prekininogen mRNA.

The elevation of plasma angiotensinogen concentration after induction of inflammation was rapid and short-lived in comparison to that of plasma kininogen concentrations (Fig. 1), suggesting some differences in regulatory processes for induction by acute inflammation. As already confirmed by other investigators [15, 16], the adrenalectomy produced a significant reduction of plasma angiotensinogen levels. The reduced levels of plasma angiotensinogen in adrenalectomized rats increased following the injection of the glucocorticoid, dexamethasone, but not of LPS or the mineralocorticoid, aldosterone. Thus, the induction of plasma angiotensinogen following acute inflammation required the intact adrenal gland. In addition to this evidence, plasma angiotensinogen levels increased in both intact and adrenalectomized rats following administration of dexamethasonem and plasma corticosterone concentrations increased rapidly following the injection of LPS. Therefore, it is evident that the induction of angiotensinogen responding to acute inflammation was mediated by glucocorticoid secretion.

In contrast, plasma kininogen concentrations were increased by the sham-operation as well as by adrenalectomy. LPS injection did not produce further elevation of plasma kininogen in adrenalectomized rats. Therefore, it is obvious that the acute inflammation induced by tissue injury itself caused the induction of plasma kininogen that was independent of the adrenal gland. This is further supported by the evidence that the administration of dexamethasone, or of aldosterone, did not cause any changes in plasma kininogen levels.

The responses of angiotensinogen and kininogen to acute inflammation identify these plasma proteins as members of the class of proteins designated as "acute phase reactants" [17]. Factors released from leukocytes have been known to control much of the acute phase response [18–20], including the hepatic induction of acute phase reactants. Of these factors, the monokines, interleukin-1 and hepatocyte-stimulating factor, have been defined as regulators of hepatic synthesis of acute phase reactants [21–23]. In addition to leukocyte factors, glucocorticoid has been thought to be involved in the induction of certain acute phase reactants [24, 25]. For example, α_1-acid glycoprotein, a typical acute phase reactant of rat, is known to be induced by glucocorticoid *in vivo* and *in vitro* [26–28]. This protein, however, is induced also by turpentine-induced acute inflammation in adrenalectomized rats [26, 28], indicating independent regulatory processes for the induction by glucocorticoid and that by mediators of the acute phase response. Recently, Woloski *et al.* [29] reported that the monokines, interleukin-1 and hepatocyte-stimulating factor, have the ability to stimulate pituitary cells to release adrenocorticotropic hormone (ACTH) and, subsequently, increase glucocorticoid secretion. Besedovsky *et al.* [30] demonstrated that the administration of interleukin-1 to mice and rats increases blood levels of ACTH and glucocorticoids. The importance of the pituitary-adrenal axis in the regulation of plasma angiotensinogen concentration was demonstrated by Hasegawa *et al.* [16] from the evidence that the decrease in plasma angiotensinogen concentration in hypophysectomized or adrenalectomized rats was prevented by the administration of ACTH or cortisol respectively. Thus, it may be possible that the induction of angiotensinogen by acute inflammation is a type of acute phase response being mediated by these monokines through the activation of the pituitary-adrenal axis. In contrast, the acute phase response of kininogen seems to be another type that is not dependent on glucocorticoid secretion. A recent report from our laboratory demonstrated that the medium conditioned by inflammatory leukocytes stimulates T-kininogen synthesis by cultured hepatocytes [31], indicating that the acute phase response of T-kininogen is inducible by a direct action on hepatocytes of factor(s) from leukocytes. All this evidence suggests that the inductions of angiotensinogen and T-kininogen are regulated by different processes. Further studies are needed to elucidate which kind of leukocyte factor(s) is involved in the control of hepatic syntheses of angiotensinogen and T-kininogen.

Recently, kininogens including T-kininogen were demonstrated to be potent inhibitors of cysteine proteinases [32, 33]; angiotensinogen is also a protein that is structurally related to the α_1-antitrypsin family [34, 35]. The importance of these facts in the inhibitor defense system is still unknown. The roles of angiotensinogen and kininogen in blood pressure regulation are also of interest. Many investigators

have provided evidence that angiotensinogen is rate-limiting in the generation of angiotensin I [36]. High levels of plasma angiotensinogen have been implicated in hypertension induced by oral contraceptives or Cushing's disease [36]. Kininogen is a component of the kallikrein–kinin system, which has been established as another system in blood pressure regulation. T-kinin is a vasoactive peptide as potent as bradykinin [37], and the presence of free T-kinin has been demonstrated in blood and inflammatory fluid of rats [38]. From these view points, the acute phase mediators including interleukin-1 may be regarded as regulators of the inhibitor defense system as well as of the blood pressure system.

REFERENCES

1. D. A. Tewksbury, *Fed. Proc.* **42**, 2724 (1983).
2. H. Kato, S. Nagasawa and S. Iwanaga, *Meth. Enzym.* **80**, 172 (1981).
3. H. Okamoto and L. M. Greenbaum, *Life Sci.* **32**, 2007 (1983).
4. H. Okamoto and L. M. Greenbaum, *Biochem. biophys. Res. Commun.* **112**, 702 (1983).
5. A. Barlas, H. Okamoto and L. M. Greenbaum, *Biochem. biophys. Res. Commun.* **129**, 280 (1985).
6. R. Kageyama, N. Kitamura, H. Ohkubo and S. Nakanishi, *J. biol. Chem.* **260**, 12060 (1985).
7. R. Kageyama, H. Ohkubo and S. Nakanishi, *Biochem. biophys. Res. Commun.* **129**, 826 (1985).
8. T. Matoba, K. Murakami and T. Inagami, *Biochim. biophys, Acta* **526**, 560 (1978).
9. T. L. Goodfriend, L. Levine and G. D. Fasman, *Science* **144**, 1344 (1964).
10. F. C. Greenwood and W. M. Hunter, *Biochem. J.* **89**, 114 (1963).
11. H. S. Schiller and M. A. Brammall, *Steroids* **24**, 665 (1974).
12. R. H. Silber, in *Methods of Biochemical Analysis* (Ed. D. Glick), Vol. 14, p. 63. Interscience Publishers, New York (1967).
13. O. M. Helmer and R. S. Griffith, *Endocrinology* **49**, 154 (1951).
14. V. J. Dzau and H. C. Herrmann, *Life Sci.* **30**, 577 (1982).
15. O. Carretero and F. Gross, *Am. J. Physiol.* **213**, 695 (1967).
16. H. Hasegawa, A. Nasjletti, K. Rice and G. M. C. Masson, *Am. J. Physiol.* **225**, 1 (1973).
17. A. Koji, in *Structure and Function of Plasma Proteins* (Ed. A. Allison), Vol. 1, p. 73. Plenum Publishing, London (1974).
18. R. G. Rupp and G. M. Fuller, *Expl. Cell Res.* **118**, 23 (1979).
19. J. D. Sipe, S. N. Vogel, J. L. Ryan, K. P. W. J. McAdam and D. L. Rosenstreich, *J. exp. Med.* **150**, 597 (1979).
20. D. L. Bornstein, *Ann. N.Y. Acad. Sci.* **389**, 323 (1982).
21. E. Tatsuda, J. D. Sipe, T. Shirahama, M. Skinner and A. S. Cohen, *J. biol. Chem.* **258**, 5414 (1983).
22. D. G. Ritchie and G. M. Fuller, *Ann. N.Y. Acad. Sci.* **408**, 490 (1983).
23. B. M. R. N. J. Woloski and G. M. Fuller, *Proc. natn. Acad. Sci. U.S.A.* **82**, 1443 (1985).
24. W. G. Heim and S. R. Ellenson, *Nature, Lond.* **213**, 1260 (1967).
25. G. Grieninger, K. M. Hertzberg and J. Pindyck, *Proc. natn. Acad. Sci. U.S.A.* **75**, 5506 (1978).
26. H. Bauman, G. L. Firestone, T. L. Burgess, K. W. Gross, K. R. Yamamoto and W. A. Held, *J. biol. Chem.* **258**, 563 (1983).
27. R. F. Feinberg, L-H. K. Sun, C. P. Ordahl and F. R. Frankel, *Proc. natn. Acad. Sci. U.S.A.* **80**, 5042 (1983).
28. A. B. Kulkarni, R. Reinke and P. Feigelson, *J. biol. Chem.* **260**, 15386 (1985).
29. B. M. R. N. J. Woloski, E. M. Smith, W. J. Meyer, G. M. Fuller and J. E. Blalock, *Science* **230**, 1035 (1985).
30. H. Besedovsky, A. Rey, E. Sorkin and C. A. Dinarello, *Science* **233**, 652 (1986).
31. N. Itoh, T. Toyohama, H. Okamoto, H. Kawano, T. Mayumi and T. Hama, *Inflammation* **11**, 345 (1987).
32. I. Ohkubo, K. Kurachi, T. Takasawa, H. Shiokawa and M. Sasaki, *Biochemistry* **23**, 569 (1984).
33. T. Sueyoshi, K. Enjyoji, T. Shimada, H. Kato, S. Iwanaga, Y. Bando, E. Kominami and N. Katunuma, *Fedn Eur. Biochem. Soc. Lett.* **182**, 193 (1985).
34. R. F. Doolittle, *Science* **222**, 417 (1983).
35. R. Kageyama, H. Ohkubo and S. Nakanishi, *Biochemistry* **23**, 3603 (1984).
36. I. A. Reid, B. J. Morris and W. F. Ganong, *A. Rev. Physiol.* **40**, 377 (1978).
37. H. Okamoto and L. M. Greenbaum, *Biochem. Pharmac.* **32**, 2637 (1983).
38. A. Barlas, K. Sugio and L. M. Greenbaum, *Fedn Eur. Biochem. Soc. Lett.* **190**, 268 (1985).