Biochimica et Biophysica Acta, 980 (1989) 299–304
Elsevier

BBA 74387

Identification of receptors in the liver that mediate endocytosis of circulating tissue kallikreins

Maria Kouyoumdjian [1], D.R. Borges [2], E.S. Prado [1] and J.L. Prado [1]

Departments of [1] Biochemistry and [2] Medicine, Escola Paulista de Medicina, São Paulo (Brazil)

(Received 22 September 1988)
(Revised manuscript received 18 January 1989)

Key words: Kallikrein; Endocytosis; Receptor; (Rat liver)

The liver plays an important role in the clearance, by receptor-mediated endocytosis, of circulating glycoproteins. It has been demonstrated that tissue kallikreins, which are acid glycoproteins, circulate in plasma, where they are poorly inhibited by plasma proteins. We have shown that the liver is the main organ that clears tissue kallikreins from the circulation. We now report the identification of receptors involved in this clearance. Using a perfused rat-liver system, and as models, pig pancreatic (PPK) and horse urinary (HoUK) kallikreins, we have found that: (a) the binding of PPK to the perfused liver was inhibited by 50 mM methyl α-D-mannoside and 20 μM mannan, was partially inhibited by 50 mM mannose and was unaffected by 1.5 μM asialofetuin; (b) binding of HoUK to the perfused liver was inhibited by 1.5 μM asialofetuin, 50 mM galactose and 50 mM lactose and was unaffected by 50 mM mannose; (c) the clearance rate of both kallikreins followed the equation $y = a \cdot x^b$; (d) their binding was Ca^{2+}-dependent and their clearance was inhibited by 3 mM chloroquine and 10 mM methylamine. Using isolated liver cells and tritiated HoUK, we calculated that 500 000 receptors/cell were present and the Scatchard plot showed that there were two apparent affinity constants: $0.24 \cdot 10^9$ l/M)(high-affinity) and $0.3 \cdot 10^8$ l/M (low-affinity). These results show that PPK is recognized by a liver mannose receptor and HoUK by the galactose receptor. The liver uptake of native and circulating tissue kallikreins thus emerges as a mechanism by which their levels in plasma are regulated.

Introduction

Tissue kallikreins are glycoproteins which act as serine proteinases and exhibit a strict specificity in their proteolytic actions [1,2]. They are characterized by their ability to release vasoactive peptides (kinins) from kininogens. These enzymes are implicated in the regulation of blood flow and in water and electrolyte balance [3]. Although the tissue kallikreins, by definition, hydrolyse kininogens of plasma to yield kinins, it has become clear that their physiologically significant proteolytic action is unrelated to or independent of this property [4]. They are found in the tissue and secretions of exocrine glands, as well as in kidney and urine. Tissue kallikreins, at least in humans, are identical in their protein part; kallikreins from different tissues are assumed to vary in their carbohydrate moieties. Their presence in plasma has already been demonstrated and a physiological role in the control of blood circulation has been suggested [5]. Although tissue kallikreins are found in blood plasma, they are innefficiently inhibited by plasmatic inhibitors [6]. Borges et al. [7,8] have shown that, in vivo, the liver is the main organ to clear tissue kallikreins from circulation and that native HoUK as well as native PPK are cleared by perfused rat liver. These two tissue kallikreins, which have similar amino-acid composition and catalytic properties, show differences in their carbohydrate content [9,10]. Using these two enzymes as models, we now report the identification of receptors involved in the liver clearance of circulating tissue kallikreins.

Abbreviations: HoUK, horse urinary kallikrein; PPK, pig pancreatic kallikrein; BSA, bovine serum albumin; EGTA, ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; SDS, sodium dodecylsulphate; pNA, *p*-nitroanilide.

Correspondence: D.R. Borges, Caixa Postal 20 239, 04034 São Paulo, Brazil.

Materials and Methods

Materials

The following materials were obtained from commercial sources: BSA (fraction V), fetuin, neuraminidase, α-L(−)- and α-D(+)-fucose, D(+)- and L-galactose, α-

D(+)- and β-D(+)-glucose, D(+)-mannose, melibiose, mannan, *N*-acetylgalactosamine, *N*-acetylglucosamine, methyl α-D-mannoside, lactose and methylamine hydrochloride (Sigma Chemical Co.); glutaraldehyde (Merck); HD-Val-Leu-Arg-pNA (S-2266) (Kabivitrium, Sweden). Acetyl-Phe-Arg-pNA was synthesized and purified by Dr. L. Juliano Neto, Department of Biophysics, Escola Paulista de Medicina, São Paulo, Brazil and chloroquine was from Fundacão do Remedio Popular, São Paulo, Brazil. Asialofetuin was obtained, from fetuin, as described in Ref. 11. PPK (1168 kU/mg protein) was obtained from Bayer Werb Elberfeld (lot SMU 2564 II) and contained a mixture of the two forms (A and B) of the β enzyme. The forms A and B contain 5.6 and 11.5% sugar composed of (residues/mol): galactose (0.75 and 1.5), *N*-acetylglucosamine (3.8 and 9.3), mannose (3.2 and 5.6) and fucose (0.51 and 1.1), respectively. HoUK was purified as described in Ref. 12 and contains 15.9% sugar composed of (residues/mol): galactose (5.2), *N*-acetylgalactosamine (10.3), mannose (4.1), fucose (1.2), *N*-acetylglucosamine (3.6) and *N*-acetylneuraminic acid (2.0). In the liver perfusion experiments, a preparation of HoUK with a specific activity of 1.4 U Ac-Phe-Arg-pNA/mg protein was used; a homogeneous preparation (SDS/PAGE with reduction at pH 8.7 and pH 4.8) with a specific activity of 3.7 U Ac-Phe-Arg-pNA/mg protein was tritiated [7]. The specific radioactivity was 0.7 mCi/mg protein and this [^{3}H]HoUK was used in the binding assay with isolated cells. The SDS-PAGE and autoradiography of [^{3}H]HoUK showed a single band; preliminary experiments showed that labelled and unlabelled HoUK were similarly cleared by perfused rat liver.

Liver perfusion in situ

The rat liver was perfused at 18°C as previously described in Ref. 13 with a flow rate of 28–30 ml/min and a consequent perfusion pressure of 12–16 cm of water. Following exsanguination with 200 ml of Krebs-Henseleit bicarbonate solution (pH 7.4) the livers of 280–330 g Wistar albino rats were perfused with 30 ml of recirculating Krebs' solution containing 1 mg/ml BSA. After 2 min of recirculation, the peristaltic pump was turned off and the desired kallikrein added to the perfusion medium in its reservoir; after 1 min of homogenization and collection of aliquot '0', the perfusion was reinitiated. Some substances (carbohydrates, proteins) were added to or omitted from the perfusion medium prior to the addition of the desired kallikrein. Aliquots of the perfusate were then collected (between 2 and 80 min); after centrifugation, the amidolytic activity was determined and the clearance of the ligand calculated. When EDTA was added to the perfusion medium, a 'Krebs-Henseleit bicarbonate' solution prepared without Ca^{2+} was used.

Amidolytic assay

The amidolytic activity of perfusates were assayed by incubating aliquots (50–100 μl) at 37°C in a final vol of 0.2 ml of 0.05 M Tris-HCl (pH 9.0)/1.0 mM EDTA [14]. Two chromogenic substrates were used: HD-Val-Leu-Arg-pNA (0.35 mM) for PPK assays and Ac-Phe-Arg-pNA (0.35 mM) for HoUK. The reactions were stopped by adding 0.8 ml 15% acetic acid and the products were measured at 405 nm. 1 U corresponded to the release of 1 μmol of *p*-nitroaniline per min.

Isolated hepatic cells

Prefixed hepatic cells (about 95% hepatocytes) were obtained after perfusion of the exsanguinated rat liver with 0.7% (v/v) glutaraldehyde as described in Ref. 15.

Binding assay

Prefixed and isolated cells ($0.5 \cdot 10^6$ cells) were incubated at room temperature for 60 min in a final vol of 0.5 ml Krebs-Henseleit bicarbonate (pH 7.4) containing 1 mg/ml BSA with [^{3}H]HoUK (96 ng, 3 pmol), after a pre-incubation period of 30 min in the presence of inhibitors. The tubes were then centrifuged at 10000 × *g* for 3 min, the supernatants were discarded and the cells washed once with 0.5 ml of Krebs-Henseleit bicarbonate/BSA. The supernatants were discarded and the cells transferred to scintillation fluid and the [^{3}H]HoUK bound to hepatic receptors was determined in a LS-100 Beckman scintillation counter. Nonspecific binding of [^{3}H]HoUK was obtained by using a Krebs-Henseleit bicarbonate solution prepared without Ca^{2+} and containing 1.0 mM EDTA.

Results

Liver perfusion

The clearance of both tissue kallikreins occurred in two phases: a rapid uptake was followed by a slow one (Fig. 1). To better characterize this behaviour, we analysed the clearance rate data by four equations: linear, logarithmic, exponential and potential. The best computer fitting of experimental data was given by the potential equation $y = a \cdot x^b$ (Table I). The uptake of both tissue kallikreins employed was inhibited by the addition of EDTA, chloroquine and methylamine to the perfusion medium but unaffected by the inclusion of excess of fetuin. The uptake of HoUK was almost completely inhibited by asialofetuin, galactose and lactose. The uptake of PPK was not affected by asialofetuin and was only weakly inhibited by galactose; this uptake was, however, inhibited by mannose, by methyl α-D-mannoside and by mannan (Table II).

Isolated cells

The binding of tritiated HoUK to prefixed and isolated hepatocytes was inhibited (two experiments) by

TABLE I

Experimental and calculated (by the equation $y = a \cdot x^b$) clearance rates of HoUK and PPK by perfused rat liver

The initial concentrations of the enzymes in the perfusion medium were 236 nM (HoUK) and 38.5 nM (PPK). The coefficients of determination, r^2, for the potential equation $y = a \cdot x^b$ were 0.999 and 0.997 for HoUK and PPK, respectively. The number of experiments were five for HoUK and four for PPK.

Perfusion time (min)	Clearance rate (pmol/min per g liver)			
	HoUK		PPK	
	experimental (mean ± S.E.)	calculated	experimental (mean ± S.E.)	calculated
2	–	–	8.3 (±0.6)	8.1
10	28.7 (±0.6)	28.5	2.6 (±0.2)	2.8
20	18.1 (±0.5)	18.1	1.8 (±0.1)	1.8
40	11.2 (±0.6)	11.4	1.2 (±0.02)	1.1
80	7.4 (±0.3)	7.4	0.7 (±0.02)	0.7

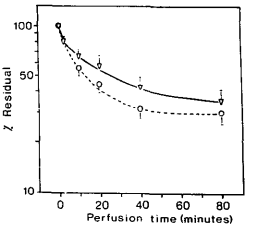

Fig. 1. Tissue kallikreins clearance by perfused rat liver. HoUK (○) and PPK (▽) were perfused by rat liver at 18°C with an initial concentration of 236 nM and 38.5 nM, respectively. Aliquots of the perfusate were taken at determined times (between 0 and 80 min) and their amidolytic activity assayed. Each point represents the mean of at least three experiments and the bar its standard error.

excess of unlabelled HoUK as well as by 1 mM EDTA. In the presence of unlabelled HoUK, the nonspecific binding of tritiated HoUK was 16%. The binding characteristics of tritiated HoUK to liver cells are shown in Table III: D-galactose, α-D(+)-fucose and melibiose were the most effective inhibitors tested. *N*-Acetylgalactosamine and asialofetuin also inhibited binding, whereas α-L(−)-fucose and L-galactose partially inhibited the binding of [^{3}H]HoUK to the isolated hepatic cells. From the other carbohydrates tested, *N*-acetylglucosamine, mannose, methyl α-D-mannoside, α-D(+)- and β-D(+)-glucose, all used at 0.1 M, inhibited less than 15% of the binding of [^{3}H]HoUK to the hepatic cells.

Kinetics of binding

The maximum amount of binding of [^{3}H]HoUK was achieved with approx. 13.3 ng (0.42 pmol) (Fig. 2A). The Scatchard plot (Fig. 2B) of these data was best interpreted as curvilinear (correlation coefficient of 0.996), suggesting the presence of at least two classes of binding site with different affinities for HoUK. The apparent affinity constants calculated were $0.24 \cdot 10^9$ 1/M (high-affinity) and $0.3 \cdot 10^8$ 1/M (low-affinity). The

TABLE II

Effect of various additions on the binding of HoUK and PPK to perfused rat liver

HoUK (236 nM) and PPK (38.5 nM) were perfused through the rat liver at 18°C at a flow rate of 28–30 ml/min; aliquots of the perfusate were collected between 0 and 80 min. The amidolytic activity determined after a 20 min incubation at 37°C, using Ac-Phe-Arg-pNA (0.35 mM) and HD-Val-Leu-Arg-pNA (0.35 mM) as substrates for HoUK and PPK, respectively (see Materials and Methods).

Additions to the perfusion medium	Molar concentration	HoUK		PPK	
		number of observations	%inhibition [a]	number of observations	%inhibition [a]
None (control)	–	4	0	4	0
EDTA	$1 \cdot 10^{-3}$	2	96	3	90
Chloroquine	$3 \cdot 10^{-3}$	2	56	1	19
Methylamine	$1 \cdot 10^{-4}$	1	31	1	66
Fetuin	$1.5 \cdot 10^{-6}$	1	0	2	0
Asialofetuin	$1.5 \cdot 10^{-6}$	3	72	2	0
D-Galactose	$5 \cdot 10^{-2}$	3	76	2	26
Mannose	$5 \cdot 10^{-2}$	1	5	3	66
Mannan	$2 \cdot 10^{-5}$	n.d. [b]	–	2	83
Lactose	$5 \cdot 10^{-2}$	1	87	n.d.	–
N-Acetylglucosamine	$5 \cdot 10^{-2}$	n.d.	–	2	32
Methyl α-D-mannoside	$5 \cdot 10^{-2}$	n.d.	–	2	86

[a] The percentage of inhibition was calculated on the basis of the binding of kallikrein in the absence of inhibitors, and was considered significant when greater than 15% (In the four control perfusions the variation of the kallikrein clearance was less than 15%.)

[b] n.d., not determined.

number of receptors on the isolated hepatic cells was calculated to be 500000 per cell, corresponding to 400000 binding sites of low affinity and to 100000 sites of high affinity.

Discussion

Many circulating ligands, including glycoproteins, are cleared from circulation by the liver through receptor-mediated endocytosis [16]. This process has been recognized as a general specific mechanism by which ligands bound to receptors are transported from the cell surface to its interior (endocytic compartments) [17]. The uptake of glycoproteins involves the recognition of specific sugars by receptors localized in the plasma membrane [18]. The galactose or asialoglycoprotein receptor is the most studied receptor in mammalian liver and less is known about the other carbohydrate-specific receptors specific for mannose/*N*-acetylglucosamine, fucose and mannose 6-phosphate [19,20]. Recently, Borges et al. [8] have observed that various tissue kallikreins perfused into rat liver were cleared in their native form, i.e., without any ex-vivo modification, such as removal of terminal sialic acid. In the present paper we have studied some characteristics of the uptake of two tissue kallikreins by the perfused rat liver as well as by isolated liver cells. The liver was perfused at 18 °C at

TABLE III

Binding characteristics of ^{3}H-HoUK to isolated hepatic cells

The incubations, with 96 nM [^{3}H]HoUK and 500000 cells, were done in duplicate and the values represent the average of two or three experiments. Two controls were always carried out: one in which only [^{3}H]HoUK was added (maximum binding) and another which contained [^{3}H]HoUK and 1 mM EDTA (nonspecific binding). The specific binding was obtained by subtracting the nonspecific of the maximum binding; the value obtained for the specific binding, considered as 100%, was used to calculate the percentage of inhibition.

Addition to the incubation medium	Molar concentration	%Inhibition
Asialofetuin	$4 \cdot 10^{-6}$	84
N-Acetylgalactosamine	$1 \cdot 10^{-1}$	82
D-Galactose	$1 \cdot 10^{-3}$	0
	$1 \cdot 10^{-2}$	51
	$5 \cdot 10^{-2}$	100
L-Galactose	$1 \cdot 10^{-4}$	19
	$1 \cdot 10^{-3}$	35
	$1 \cdot 10^{-2}$	36
	$1 \cdot 10^{-1}$	40
Melibiose	$1 \cdot 10^{-3}$	0
	$1 \cdot 10^{-2}$	44
	$1 \cdot 10^{-1}$	100
α-L(−)-Fucose	$1 \cdot 10^{-2}$	0
	$1 \cdot 10^{-1}$	43
α-D(+)-Fucose	$1 \cdot 10^{-4}$	45
	$1 \cdot 10^{-3}$	52
	$1 \cdot 10^{-2}$	75
	$1 \cdot 10^{-1}$	100

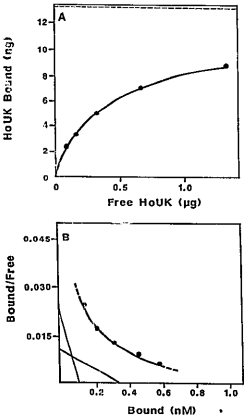

Fig. 2. Binding of HoUK to isolated hepatic cells. Incubations were carried out in the presence of [^{3}H]HoUK (6–98 nM) and 500000 cells, in a final vol of 0.5 ml of Krebs-Henseleit bicarbonate/BSA. Each point represents the mean of duplicate determinations. Binding data were analysed by a computer program; the coefficient of correlation was 0.996 and the standard error of the analysis was 0.0054. (A) Saturation curve of [^{3}H]HoUK binding to isolated hepatic cells at room temperature. The specific binding was calculated considering the nonspecific binding that occurs in the calcium-free medium (see Materials and Methods); in this situation less than 20% of labelled kallikrein binds to the liver cells. (B) The Scatchard plot of these data. The association constants (K_a) and binding site capacities for the high- and low-affinity [^{3}H]HoUK binding sites were: $0.24 \cdot 10^9$ l/M, and $0.09 \cdot 10^{-12}$ M, and $0.3 \cdot 10^8$ l/M and $0.33 \cdot 10^{-12}$ M, respectively.

which point the intact organ is capable of binding and internalizing but not degrading most protein ligands [20]. The liver clearance rate of both tissue kallikreins were biphasic and concentration-dependent, as observed earlier for rat plasma kallikrein [11,21], and can be explained by a potential equation. The binding of both tissue kallikreins to the perfused rat liver was Ca^{2+}-dependent, differing from the clearance of plasma kallikrein which is Ca^{2+}-independent [11]. We observed that chloroquine and methylamine did not inhibit the uptake but presumably did inhibit intracellular pathways of both tissue kallikreins. These lysosomotropic agents are known to increase the pH inside the endocytic compartment and lysosomes and to interfere with the galactose-receptor recycling and ligand processing and degradation [22]. These results indicate that both tissue kallikreins are cleared by the liver through a similar mechanism and that chloroquine may function as a

general inhibitor of the receptor-mediated endocytosis for glycoproteins. Our results suggest that PPK is recognized by a liver mannose receptor, since its calcium-dependent clearance was inhibited by mannose, methyl α-D-mannoside and mannan [20]. The uptake of PPK was weakly inhibited by galactose in agreement with the results for the binding of mannan to either isolated liver cells or purified mannose receptor [23]. Two systems that recognize mannose residues and clear glycoproteins from the blood are known to be operative in the rat liver: one, localized in macrophages and specialized endothelial cells, is responsible for the clearance of mannose-terminal glycoproteins [24]; the other, localized in hepatocytes, displays specificity for mannose and *N*-acetylglucosamine residues found in the 'core' region of asparagine-linked oligosaccharides and is known as core-specific lectin [25]. If the structure of the carbohydrate chain of PPK proposed by Moriya et al. [26] is correct, this glycoprotein may be recognized by the core-specific lectin. The addition of asialofetuin to the perfusion medium did not alter the clearance of PPK, indicating that the galactose receptor was not involved in the uptake of this glycoprotein. The binding characteristics of HoUK by the perfused rat liver, as well as by isolated liver cells, indicate that this native glycoprotein was recognized by the galactose receptor. Its uptake was inhibited by asialofetuin, D(+)-galactose, melibiose, α-D(+)-fucose and *N*-acetylgalactosamine but was unaffected by fetuin or mannose. These properties are similar to those described for glycoproteins which, having galactose as terminal residues, bind to the liver galactose receptor [27]. Using isolated liver cells, we observed that the binding of HoUK was a saturable and a highly specific process. Prefixing liver cells with glutaraldehyde preserves their original shape well, avoids the use of collagenase (which may impair receptor function) [15] and bypasses difficulties in quantification of receptors on the rat hepatocyte [19]. We have calculated that the number of receptors/cell was 500 000, a similar value to the one obtained by Matsuura et al. [15], who studied the binding of specific antibody to hepatic binding protein to the galactose receptor of prefixed and isolated liver cells. The Scatchard plot indicated that at least two receptor forms were present with the following affinity constants: $0.24 \cdot 10^9$ l/M (high-affinity) and $0.3 \cdot 10^8$ l/M (low-affinity). These values were similar to the affinity constants obtained by Weigel et al. [28] for the binding of asialoorosomucoid to the galactose receptor and are compatible with a physiological binding of tissue kallikreins to liver receptor. The clearance, by liver, of circulating tissue kallikreins through receptor-mediated endocytosis could eventually be an important mechanism by which their plasma levels and, therefore, their systemic actions are controlled, since their inactivation by plasma inhibitors is inefficient [6]. Indeed, in human plasma obtained from patients with liver cirrhosis, it has been recently reported that an increase in circulating tissue kallikrein occurred [29]. Although Ashwell and Harford [20] have postulated that hepatic lectins may be involved in the clearance of biologically reactive glycoproteins after they have exerted a particular physiological function, no examples of such endocytosis have been described. Receptor-mediated endocytosis of tissue kallikreins may indeed be a physiological role of the various receptor systems for circulating glycoproteins that are operative in the liver.

Acknowledgements

We would like to thank Miss Hercilia M. Molina for the technical assistance. A. Bracht (Universidade de Maringá, Brazil) and W.H. Evans (National Institute for Medical Research, U.K.) offered helpful comments on the manuscript. This work was supported by CAPES and FINEP; D.R.B. received a grant from FAPESP (87/0323-8).

References

1 Fiedler, F. (1979) in Handbook on Experimental Pharmacology, Vol. XXV Suppl. (Erdos E.G., ed.), pp. 103–161. Springer-Verlag, New York.
2 Prado, E.S., Araujo-Viel, M.S., Juliano, M.A., Juliano, N.L., Stella, R.C.R. and Sampaio, C.A.M. (1986) Biol. Chem. Hoppe-Seyler 367, 199–205.
3 Orstavik, T.B. (1980) J. Histochem. Cytochem. 28, 881–889.
4 Schachter, M. (1980) Pharmacol. Rev. 31, 1–17.
5 Rabito, S.F., Seichi, A.G., Kher, V. and Carretero, O.A. (1982) Am. J. Physiol. 242, H602–H610.
6 Chao, J., Tillmann, D.M., Wang, M., Margolius, H.S. and Chao, L. (1986) Biochem. J. 239, 325–331.
7 Borges, D.R., Sampaio, C.A.M., Llosa, P.D. and Prado, J.L. (1986) Adv. Exp. Biol. Med. 198A, 229–233.
8 Borges, D.R., Kouyoumdjian, M., Prado, E.S. and Prado, J.L. (1986) Adv. Exp. Biol. 198A, 235–239.
9 Prado, E.S., Sampaio, C.A.M., Araujo-Viel, M.S. and Stella, R.C.R. (1982) Agents Actions 9, 162–166.
10 Fiedler, F., Hirschauer, C. and Werle, E. (1975) Hoppe-Seyler's Z. Physiol. Chem. 356, 1877–1891.
11 Borges, D.R., Gordan, A.H., Guimaraes, J.A. and Prado, J.L. (1985) Braz. J. Med. Biol. Res. 18, 187–194.
12 Giusti, E.P., Sampaio, C.A.M. and Prado, E.S. (1978) Agents Actions 8, 164–165.
13 Borges, D.R., Limãos, E.A., Prado, J.L. and Camargo, A.C.M. (1976) Naunyn-Schmiedebergs Arch. Pharmacol. 295, 33–40.
14 Claeson, G., Friberger, P., Knos, M. and Eriksson, E. (1978) Haemostasis 7, 76–78.
15 Matsuura, S., Nakada, N.L., Sawamura, T. and Tashiro, Y. (1982) J. Cell Biol. 95, 864–875.
16 Goldstein, J.L., Anderson, R.G.W. and Brown, M.S. (1979) Nature 279, 679–685.
17 Evans, W.H. (1986) Biochem. Soc. Trans. 14, 170–172.
18 Maury, C.P.J. (1983) Scand. J. Gastroenterol. 18, 321–325.
19 Schwartz, A.L. (1983) CRC Crit. Rev. Biochem. 16, 207–233.
20 Ashwell, G. and Harford, J. (1982) Annu. Rev. Biochem. 51, 531–554.
21 Borges, D.R., Webster, M.E., Guimaraes, J.A. and Prado, J.L. (1981) Biochem. Pharmacol. 30, 1065–1069.

22 Schwartz, A.L., Bolognesi, A. and Fridovich, S.E. (1984) J. Cell Biol. 98, 732–738.
23 Mizuno, Y., Kozutsumi, Y., Kawasaki, T. and Yamashina, I. (1981) J. Biol. Chem. 256, 4247–4252.
24 Simmons, B.M., Stahl, P.D. and Russell, J.H. (1987) Biochem. Biophys. Res. Commun. 146, 849–854.
25 Colley, K.J. and Baenziger, J.U. (1987) J. Biol. Chem. 262, 10290–10295.
26 Moriya, H., Ikekita, M. and Kizuki, K. (1983) Adv. Exp. Biol. Med. Kinins III, Part A, pp. 309–316.
27 Stockert, R.J., Morell, A.G. and Scheinberg, I.H. (1977) Science 197, 667–668.
28 Weigel, P.H. (1980) J. Biol. Chem. 255, 6111–6120.
29 Kizuki, K., Susuki, S., Aoki, K., Kamada, M., Ikekita, M. and Moriya, H. (1987) in Kinin '87 Tokyo International Congress, Japan, 29 Nov–4 Dec, abstr. no. P-1-A13, p. 108.