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KALLIDIN AND BRADYKININ METABOLISM BY ISOLATED CEREBRAL MICROVESSELS

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Abstract—Although kinins have been reported to affect cerebral vascular tone and permeability, their actions are not potentiated by angiotensin converting enzyme inhibitors. To investigate cerebral vascular kinin metabolism, porcine cerebral microvessels were isolated by differential sieving and centrifugation and characterized by microscopic examination and marker enzyme enrichment. Purified microvessels contained a membrane-bound carboxypeptidase which hydrolyzed the C-terminal Phe-Arg bond of both kallidin and bradykinin. Hydrolysis was optimal at pH 7.0, was activated more than 300% by 0.1 mM $CoCl_2$, and was inhibited by *o*-phenanthroline and the carboxypeptidase N (EC 3.4.17.3) inhibitor DL-2-mercaptomethyl-3-guanidino-ethylthiopropanoic acid (MERGETPA) ($IC_{50} = 2\ \mu M$). Conversely, inhibitors of angiotensin I converting enzyme (captopril), neutral endopeptidase (phosphoramidon), post proline cleaving enzyme (Z-Pro-prolinal), dipeptidyl(amino)peptidase IV (diprotin A) and aminopeptidase M (amastatin) had no effect. When the rates of C-terminal hydrolysis of kallidin by detergent-solubilized cerebral microvasculature were determined over a range of substrate concentrations (16.6 to 250 μM), the K_m and V_{max} values obtained were 26.0 ± 3.0 μM and 14.7 ± 1.3 nmol/min/ml (N = 4) respectively. These data suggest that a cerebral microvascular carboxypeptidase may play a role *in vivo* in modulating the effects of kinins on cerebral blood flow and permeability and in preventing circulating kinins from crossing the blood–brain barrier.

Kallidin and bradykinin are produced by plasma and glandular kallikrein. Although capable of lowering systemic blood pressure, kinins more likely function as local hormones since they are rapidly degraded across the pulmonary circulation by angiotensin I converting enzyme (ACE; EC 3.4.15.1) [1].

Kinins dilate cerebral vessels [2–4] and have been implicated in the pathogenesis of vasogenic brain edema [5, 6]. If circulating and/or locally released kinins do modulate cerebral blood flow and permeability, the capacity of cerebral vessels to degrade them could have important physiologic significance. However, although ACE has been identified in cerebral microvessels [7, 8], ACE inhibitors such as captopril do not potentiate the effects of kinins on cerebral vessels [9–11]. These data suggest that cerebral peptidases other than ACE may be significant.

Plasma carboxypeptidase N (CPN; EC 3.4.17.3) hydrolyzes C-terminal basic amino acids from a variety of biologically active peptides including kinins, anaphylatoxins, fibrinopeptides and enkephalins [12, 13]. An enzyme immunologically related to plasma CPN has been identified on pulmonary endothelium [14], and we have demonstrated previously that the cell surface of aortic endothelium contains a CPN-like enzyme that hydrolyzes the C-termini of kinins to produce des(Arg)kinins [15]. If a CPN-like enzyme is present in cerebral microvasculature, such local kinin metabolism would not only terminate the biologic action of the classic (B_2) kinins, but would also form des(Arg)kinins, metabolites which constrict cerebral microvessels via B_1 kinin receptors [16, 17]. In view of these considerations, we examined the metabolism of kinins by isolated porcine cerebral microvessels.

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MATERIALS AND METHODS

Materials. Kallidin, bradykinin and des(Arg^9)-bradykinin, γ-gluamyl- and α-glutamyl-2-naphthylamide, *o*-phenanthroline, amastatin and phosphoramidon [*N*-(α-rhamnopyranosyl-oxyhydroxyphosphinyl-L-leucyl-L-tryptophan)] were obtained from the Sigma Chemical Co. (St. Louis, MO). The ACE inhibitor captopril and the CPN inhibitor MERGETPA (DL-2-mercaptomethyl-3-guanidino-ethylthiopropanoic acid) were obtained from Squibb (Princeton, NJ) and Calbiochem-Behring (San Diego, CA) respectively. The post proline cleaving enzyme (EC 3.4.21.26) inhibitor Z-Pro-prolinal was provided by Dr. Sherwin Wilk (Mount Sinai School of Medicine, New York, NY). The nylon sieves used for microvessel isolation were from Tetko, Inc. (Elmsford, NY). The MN 300 Uniplates used for TLC were from Analtech, Inc. (Newark, DE), and the amino acid standards, *o*-phthalaldehyde crystals and *o*-phthalaldehyde (OPA) reagent solution used for HPLC were from the Pierce Chemical Co. (Rockford, IL).

Cerebral microvessel isolation. Whole brains, obtained both frozen (Pel-Freeze; Rogers, AR) and from freshly slaughtered hogs, were immersed in cold Earle's buffer (pH 7.4) containing 0.1% (w/v) bovine serum albumin. Microvessels were prepared as previously described [8, 18] according to Brendel *et al.* [19] as modified by Selivonchick and Roots [20]. Cortical grey matter was minced and homogenized (30 sec) in 3 vol. of buffer using a Waring blender.

The extract was then homogenized (Dounce, four strokes), poured through 1000 and 300 micron mesh nylon sieves, and rehomogenized. Vessels were collected on a 44 micron mesh sieve, centrifuged (4,000 *g*, 10 min), and resuspended (0.25 M sucrose). After centrifugation (21,000 *g*, 45 min) on a discontinuous sucrose gradient (1.0 and 1.5 M), the pellet was freeze-powdered, sonicated and repelleted (20,000 *g*, 15 min). Activity was measured either directly or after solubilization with 5% (v/v) Triton X-100. Microvessel purity was determined by phase contrast microscopy and assay of γ-glutamyltransferase [8, 18].

Enzyme assays. γ-Glutamyltransferase (EC 2.3.2.2) and aminopeptidase A (EC 3.4.11.7) activities were assayed as the rates of hydrolysis of γ-glutamyl- and α-glutamyl-naphthylamide respectively [18, 21]. Specific activities are expressed as units/mg protein where one unit equals the hydrolysis of one nanomole substrate per minute. Protein was determined according to Bradford [22] using bovine serum albumin as a standard.

Kinin metabolism. Qualitative analysis of kinin metabolism was carried out by TLC on MN 300 cellulose plates [8, 15]. Quantitative analysis was performed by HPLC as described previously [15]. Microvessel carboxypeptidase activity was determined as the rate of MERGETPA-sensitive C-terminal Arg released from kallidin and bradykinin in the presence of 0.1 mM $CoCl_2$. The standard incubation consisted of mixing the peptide in 295 μl of 100 mM sodium phosphate buffer (pH 7.0) with 5 μl of a dilution of microvessel protein (37°). At sequential time intervals, 60-μl aliquots were immersed in a boiling water bath (5 min) to terminate the reaction, cooled on ice, and centrifuged in a Brinkmann table top centrifuge (3 min), and the supernatant fraction was collected for analysis.

For determination of pH dependence, a 100 mM sodium acetate buffer was used over the pH range 4.0 to 6.0, and the above 100 mM phosphate buffer was used from pH 6.5 to 8.5. For inhibition studies, inhibitors were preincubated with enzyme and buffer for 20 min at 37°. For K_m determinations, measurements of the initial velocity of hydrolysis were determined over a range of substrate concentrations (16.6 to 250 μM). Data were plotted as $1/V$ vs $1/S$ and fit to the best straight line.

A high performance liquid chromatograph system (Waters Associates; Milford, MA) consisting of two model 6000A pumps, a model 730 data module, a model 721 system controller, a model 712B WISP™ Autosampler and a model 420 fluorescence detector was employed for the HPLC analysis. Standards and unknowns (10–60 μl) were automatically derivatized with OPA solution (20 μl) 3 min prior to chromatography (Precolumn Derivatization Program-Waters 710B WISP™ Autosampler) and subsequently separated on a reverse phase column (Waters, 10 micron, C_{18}-Radial-PAK™, 8 mm × 10 cm) at a constant flow rate of 5.0 ml/min utilizing a linear gradient from 100% Buffer A to 40% Buffer A/60% Buffer B. Integration of sample peak areas and quantitation of metabolites against the last-run standard were automatically calculated by the data module. Standards were run every sixth injection.

The OPA solution was made fresh daily by mixing 3.8 ml *o*-phthalaldehyde reagent solution, 0.1 ml methanol, 0.1 ml β-mercaptoethanol and 16.8 mg *o*-phthalaldehyde crystals. Buffer A was 10 mM sodium phosphate (pH 7.0) and Buffer B was a 50/50 (v/v) mixture of Buffer A and acetonitrile.

RESULTS

Isolated microvessels. As previously found [8, 18], isolated cerebral microvessels (7–70 microns diameter) exhibited extensive dichotomous branching and numerous bifurcations. Endothelial cell nuclei could be visualized, whereas little or no non-vascular contamination could be detected.

The extent of microvessel purification was determined by enrichment of γ-glutamyltransferase activity. As seen in Table 1, cerebral microvessel γ-glutamyltransferase activity (0.96 ± 0.16 nmol/min/mg) was enriched 24-fold over original homogenate (0.04 ± 0.01 nmol/min/mg). As previously found [18], microvessels were also enriched (16.3-fold) in aminopeptidase A α-glutamyl-2-naphthylamidase activity. Homogenate and microvessel activities were 0.30 ± 0.04 and 4.9 ± 0.7 nmol/min/mg respectively.

Microvessel kinin metabolism. In the presence of 10 μM amastatin to inhibit microvessel aminopeptidase A (EC 3.4.11.7) [18], microvessels were

Table 1. Distribution of α-glutamyltransferase and aminopeptidase A in homogenates of whole brain and isolated cerebral microvessels

Enzyme	Specific activity (nmol substrate hydrolyzed/min/mg protein)		
	Brain homogenate	Cerebral microvessels	Enrichment
γ-Glutamyltransferase	0.04 ± 0.01	0.96 ± 0.16	24.0
Aminopeptidase A	0.30 ± 0.04	4.9 ± 0.7	16.3

Values given are the means ± SE from six experiments. Enrichment is calculated as (mean specific activity of microvessel fraction)/(mean specific activity of brain homogenate).

incubated with angiotensin I and the metabolites separated by TLC. Due to the presence of cerebral microvascular ACE [7, 8], angiotensin I was converted to products which co-migrated with angiotensin II and His/Leu. In contrast, incubation of kallidin resulted in little or no C-terminal dipeptide product (Phe/Arg) and captopril (100 μM) had no effect on the observed pattern of hydrolysis. Rather, kallidin was initially hydrolyzed to produce metabolites co-migrating with Lys and Arg. In the presence of 10 μM amastatin to inhibit cerebral microvascular aminopeptidase M (EC 3.4.11.2) [8, 23], Lys production was inhibited completely, whereas Arg production was unaffected. C-terminal Arg production, in turn, was inhibited completely by the CPN inhibitor MERGETPA (100 μM).

Arg was the sole product produced from bradykinin. However, in contrast to the above, not all Arg production could be inhibited by MERGETPA (i.e. C-terminal hydrolysis). This observation suggested that bradykinin was being metabolized by hydrolysis of both its C-terminal Phe^8-Arg^9 bond by a MERGETPA-sensitive carboxypeptidase and its N-terminal Arg^1-Pro^2 bond by a MERGETPA-insensitive peptidase. Consistent with this interpretation, incubation of des(Arg^9)bradykinin resulted in the gradual production of a single detectable product (N-terminal Arg) which was not inhibited by MERGETPA.

Kinin C-terminal hydrolysis. Since C-terminal hydrolysis was the predominant metabolic reaction observed, quantitative studies using HPLC were conducted to determine whether the MERGETPA-sensitive carboxypeptidase was enriched in cerebral microvasculature. Control studies of original homogenate and microvessel C-terminal hydrolysis of kallidin and bradykinin (Arg production inhibited by 0.1 mM MERGETPA) established that the rates of production of C-terminal Arg were directly proportional to both time of incubation (0–270 min) and amount of protein used. Microvessel hydrolysis of the Phe^8-Arg^9 bond of bradykinin (0.77 ± 0.11 nmol/min/mg) was enriched 5.1-fold over that in original homogenate (0.15 ± 0.06 nmol/min/mg). A comparable enrichment was also found for microvessel C-terminal hydrolysis of kallidin (carried out in the presence of 10 μM amastatin to prevent hydrolysis of N-terminal Lys).

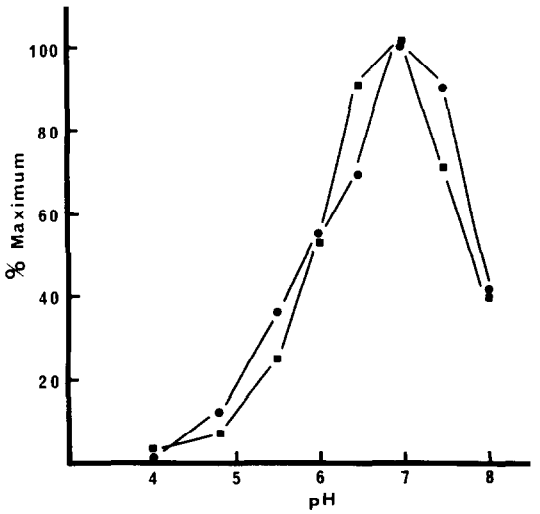

Fig. 1. Effect of pH on the C-terminal hydrolysis of the Phe-Arg bond of kallidin (●) and bradykinin (■) by cerebral microvessels. Incubations contained 0.1 mM $CoCl_2$ and 10 μM amastatin. Values shown are single (kallidin) or the average of two (bradykinin) determinations and are expressed as percent maximal activity (9.7 and 9.0 nmol/min/ml respectively).

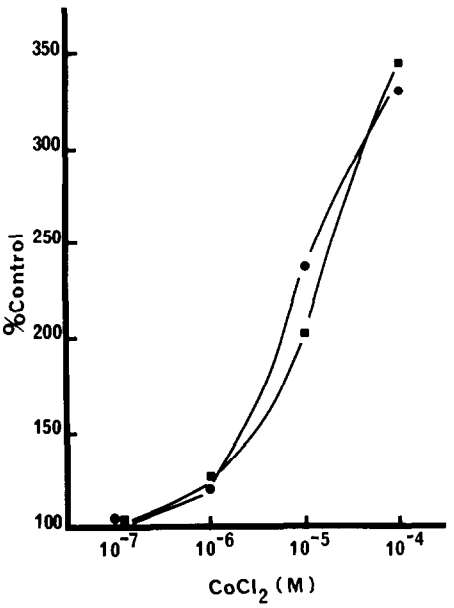

Fig. 2. Activation of cerebral microvessel C-terminal hydrolysis of the Phe-Arg bond of kallidin (●) and bradykinin (■) by $CoCl_2$. Incubations contained 10 μM amastatin. Values shown are single (kallidin) or the average of three (bradykinin) determinations and are given as percent control activity in the absence of $CoCl_2$ (2.5 and 3.7 nmol/min/ml respectively).

After centrifugation (20,000 g, 15 min) of the freeze-powdered microvessels, more than 70% of the MERGETPA-sensitive C-terminal hydrolytic activity was found in the pelleted membrane fraction. C-terminal hydrolysis of bradykinin and kallidin (in the presence of amastatin) was optimal at pH 7.0 (Fig. 1) and was activated more than 300% by 0.1 mM $CoCl_2$ (Fig. 2). In contrast, no significant activation was produced by NaCl (less than 20% change at 1 mM final concentration). Similar results were obtained using detergent-solubilized microvessels.

C-terminal hydrolysis of kallidin was inhibited 100% by 1 mM *o*-phenanthroline and by the CPN inhibitor MERGETPA (IC_{50} = 2 μM) (Fig. 3). Bradykinin hydrolysis was similarly inhibited (Fig. 3) although, as expected from the earlier TLC studies, complete inhibition of Arg production could not be achieved (≅ 80%) even in the presence of 100 μM MERGETPA due to hydrolysis of the N-terminal

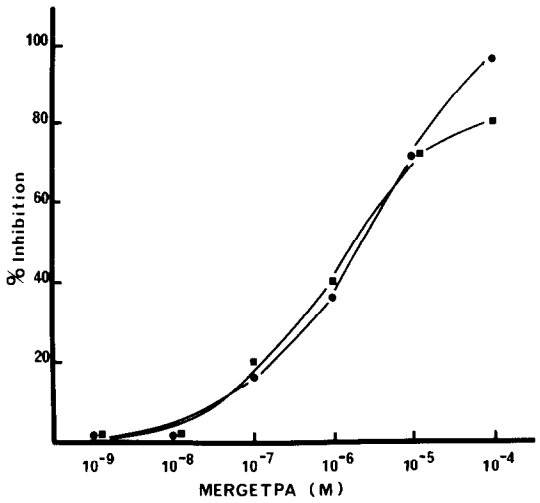

Fig. 3. Inhibition (%) of cerebral microvessel C-terminal hydrolysis of the Phe-Arg bond of kallidin (●) and bradykinin (■) by a range of concentrations of MERGETPA. Incubations contained 0.1 mM $CoCl_2$ and 10 μM amastatin. Values shown are averages of two (kallidin) or three (bradykinin) determinations. Control values in the absence of MERGETPA for kallidin and bradykinin were 9.7 and 11.5 nmol/min/ml respectively.

Arg^1-Pro^2 bond. Finally, in contrast to the inhibition by MERGETPA of C-terminal hydrolysis, inhibitors of ACE (captopril), neutral endopeptidase (phosphoramidon), post proline cleaving enzyme (Z-Pro-prolinal) and dipeptidyl(amino)peptidase IV (diprotin A) had no effect (less than 10% inhibition at 10 μM final concentration).

When the rate of C-terminal hydrolysis of kallidin by detergent solubilized membrane was determined over a range of substrate concentrations (16.6 to 250 μM) (Fig. 4; individual experiment), the K_m and V_{max} values obtained were 26.0 ± 3.0 μM and 14.7 ± 1.3 nmol/min/ml (N = 4) respectively.

Kinin N-terminal hydrolysis. Since the Arg^1-Pro^2 bond of both bradykinin and des(Arg^9)bradykinin were also slowly hydrolyzed, preliminary localization and characterization studies of this N-terminal hydrolytic activity were conducted. The rates of original homogenate and microvessel N-terminal hydrolysis of des(Arg^9)bradykinin were proportional to both incubation time and protein. However, in contrast to the C-terminal hydrolysis noted above, microvessel N-terminal hydrolysis of des(Arg^9)-bradykinin (0.41 ± 0.08 nmol/min/mg) was not enriched over that in original homogenate (0.40 ± 0.05 nmol/min/mg) (N = 3).

N-terminal hydrolysis was optimal at neutral pH (pH 7.0) but was unaffected by $CoCl_2$ (0.1 mM). Activity was inhibited completely by *o*-phenanthroline (1.0 mM) but, as expected from the earlier TLC studies, was unaffected by MERGETPA. Comparable results were obtained for the N-terminal hydrolysis of bradykinin (in the presence of MERGETPA to prevent C-terminal hydrolysis).

DISCUSSION

In addition to its presence in plasma, kallikrein has been reported in isolated vasculature [24] and the central nervous system [25–27]. Both infusion and topical application of kinins dilate cerebral vessels [9–11] and increase vascular permeability [5, 6]. Further, Kamitani *et al.* [28] have demonstrated that kinins produced endogeneously within the CNS can alter cerebral vascular tone. Thus, both circulating and locally produced kinins may modulate cerebral

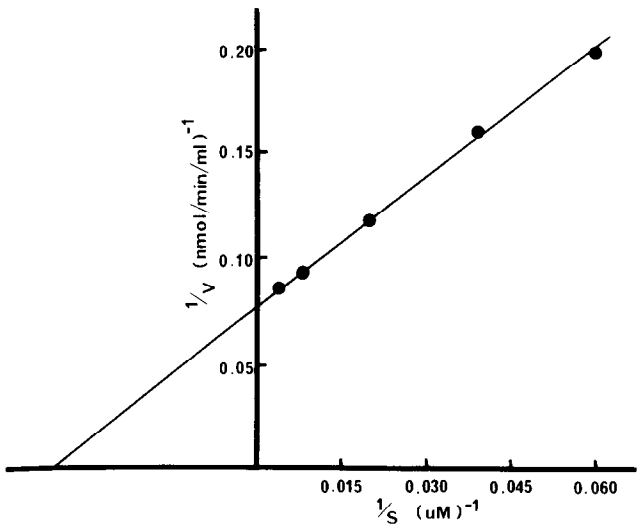

Fig. 4. Lineweaver–Burk plot of the rate of C-terminal hydrolysis of the Phe-Arg bond of kallidin by detergent-solubilized cerebral microvessel membrane. Incubations contained 0.1 mM $CoCl_2$ and 10 μM amastatin.

blood flow and contribute to the pathogenesis of cerebral edema [5, 6].

In view of the above, the metabolism of kinins by cerebral vasculature could have important physiologic significance. Cerebral microvessels contain a number of peptidases including aminopeptidase A (AmA; EC 3.4.11.7), aminopeptidase M (AmM; EC 3.4.11.2) and angiotensin I converting enzyme (ACE; EC 3.4.15.1) [7, 8, 18]. AmA converts angiotensin II to angiotensin III but does not metabolize kinins [18]. AmM converts kallidin to bradykinin but, due to the N-terminal Arg^1-Pro^2 bond, does not further hydrolyze bradykinin [23]. Thus, ACE is the only previously identified cerebral vascular peptidase capable of degrading kinins [7, 8]. However, numerous studies have demonstrated that, although ACE inhibitors block the constrictor effect of angiotensin I on cerebral arteries, they have little potentiating effect on kinins [9–11]. Thus, peptidases other than ACE may be responsible for kinin metabolism in cerebral vasculature.

Consistent with the above observations [9–11], the present data demonstrate that, at least *in vitro*, cerebral microvessel ACE does not contribute significantly to kinin metabolism. Rather, microvessels preferentially metabolized kinins by hydrolysis of the C-terminal Phe-Arg bond. Since the present study was carried out with microvessel extracts rather than purified enzyme, no firm conclusions can be drawn regarding the identity of the kinin C-terminal hydrolytic activity. However, this activity has many properties in common with plasma CPN [29] and a carboxypeptidase present on vascular endothelium [15]. The microvessels hydrolyzed a basic C-terminal amino acid (Arg) from bradykinin at a rate (0.77 nmol/min/mg) that was considerably faster than that previously found for porcine aorta (0.035 nmol/min/mg) [15]. Like purified plasma CPN [29], microvessel C-terminal hydrolysis was optimal at pH 7.0, was activated by $CoCl_2$, and was inhibited by *o*-phenanthroline and MERGETPA. In contrast, inhibitors of aminopeptidases (amastatin, diprotin A), endopeptidases (phosphoramidon, Z-Pro-prolinal) and angiotensin converting enzyme (captopril) had no effect. In addition, the affinity of kallidin for the microvascular activity ($K_m = 26\ \mu M$) was similar to that reported for an endothelial carboxypeptidase (45–60 μM) [15] and human plasma CPN (25–45 μM) [29].

The cellular and subcellular localization of this cerebral microvascular activity remains to be determined. However, an endothelial cell surface localization would seem likely considering the endothelial localization on porcine aorta [15] and the observation of Ryan and Ryan [14] that pulmonary endothelium contains a cell surface enzyme which cross-reacts with antibody to plasma CPN. Endothelial activity could modulate the effects of circulating kinins on local blood flow/permeability and, as a component of the blood–brain barrier, prevent peripheral kinins from entering the central nervous system. However, if also present within the vascular wall, metabolism of kinins produced within the CNS may also occur [28].

Cerebral vasculature has been reported to contain both B_2 [2–4] and B_1 [16, 17] kinin receptors. Kallidin and bradykinin stimulate B_2 receptors, whereas des(Arg^{10})kallidin and des(Arg^9)bradykinin act through B_1 kinin receptors [30]. Although B_1 kinin receptors are probably not present normally, they may be induced under stress and/or inflammatory conditions [30]. Thus, kinin hydrolysis by a cerebral microvascular carboxypeptidase could alter the relative balance between B_2- and B_1-mediated effects (i.e. degradation of B_2 kinin agonists and formation of B_1 kinin agonists).

In view of their possible biologic action via B_1 receptors, it will be interesting to determine how des(Arg)kinins are degraded by cerebral vasculature. The results of the present study indicate that des(Arg^9)bradykinin is primarily inactivated via hydrolysis of the N-terminal Arg^1-Pro^2 bond. However, since this N-terminal hydrolytic activity was not enriched in the microvessel preparations, further localization and characterization studies will be required.

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REFERENCES

1. R. L. Soffer, *A. Rev. Biochem.* **45**, 73 (1976).
2. N. Toda, *Am. J. Physiol.* **232**, H267 (1977).
3. M. Wahl, A. R. Young, L. Edvinsson and F. Wagner, *J. Cerebr. Blood Flow Metab.* **3**, 231 (1983).
4. E. T. Whalley and M. Wahl, *Naunyn-Schmiedeberg's Archs Pharmac* **323**, 66 (1983).
5. A. Unterberg, C. Dautermann, A. Baethmann and W. Muller-Esterl, *J. Neurosurg.* **64**, 269 (1986).
6. A. Unterberg, M. Wahl and A. Baethmann, *J. Cerebr. Blood Flow Metab.* **4**, 574 (1984).
7. P. Brecher, A. Tercyak and A. V. Chobanian, *Hypertension* **3**, 198 (1983).
8. L. Churchill, H. H. Bausback, M. E. Gerritsen and P. E. Ward, *Biochim. biophys. Acta* **923**, 35 (1987).
9. E. T. Whalley and M. Wahl, *Pflügers Archs* **398**, 175 (1983).
10. M. Wahl, A. R. Young, L. Edvinsson and F. Wagner, *J. Cerebr. Blood Flow Metab.* **3**, 339 (1983).
11. E. T. Whalley, *Naunyn-Schmiedeberg's Archs Pharmac.* **335**, 433 (1987).
12. V. A. Bokisch and H. J. Muller-Eberhard, *J. clin. Invest.* **49**, 2427 (1970).
13. R. Huey, C. M. Bloor, M. S. Kawahara and T. E. Hugli, *Am. J. Path.* **112**, 48 (1983).
14. U. S. Ryan and J. W. Ryan, *Clin. Lab. Med.* **3**, 577 (1983).
15. F. E. Palmieri, H. H. Bausback, L. Churchill and P. E. Ward, *Biochem. Pharmac.* **35**, 2749 (1986).
16. E. T. Whalley, H. Fritz and R. Geiger, *Naunyn-Schmiedeberg's Archs Pharmac.* **324**, 296 (1983).
17. E. T. Whalley, M. Wahl and C. A. M. Sampaio, *Hypertension* **5**, V-34 (1983).
18. H. H. Bausback, L. Churchill and P. E. Ward, *Biochem. Pharmac.* **37**, 155 (1988).
19. K. Brendel, E. Meezan and E. C. Carlson, *Science* **185**, 953 (1974).
20. D. P. Selivonchick and B. I. Roots, *Lipids* **12**, 165 (1977).
21. A. J. Kenny, in *Proteinases in Mammalian Cells and Tissues* (Ed. A. J. Barrett), p. 393. Elsevier/North Holland Biomedical Press, New York (1977).
22. M. Bradford, *Analyt. Biochem.* **72**, 248 (1976).
23. F. E. Palmieri, J. J. Petrelli and P. E. Ward, *Biochem. Pharmac.* **34**, 2309 (1985).

24. H. Nolly, A. G. Scicli, G. Scicli and O. A. Carretero, *Circulation Res.* **56**, 816 (1985).
25. J. Chao, C. Woodley, L. Chao and H. S. Margolius, *J. biol. Chem.* **258**, 15173 (1983).
26. G. R. Thomas, H. Thibodeaux, H. S. Margolius and P. J. Privitera, *Hypertension* **6** (Suppl. I), I-46 (1984).
27. J. A. V. Simson, R. Dom, J. Chao, C. Woodley, L. Chao and H. S. Margolius, *J. Histochem. Cytochem.* **33**, 951 (1985).
28. T. Kamitani, M. H. Little and E. F. Ellis, *Circulation Res.* **57**, 545 (1985).
29. R. A. Skidgel, A. R. Johnson and E. G. Erdos, *Biochem. Pharmac.* **33**, 3471 (1984).
30. F. Marceau, A. Lussier, D. Regoli and J. P. Giroud, *Gen. Pharmac.* **14**, 209 (1983).