Biochem. J. (1990) 271, 381–385 (Printed in Great Britain)

The hydrolysis of brain and atrial natriuretic peptides by porcine choroid plexus is attributable to endopeptidase-24.11

Anne BOURNE and A. John KENNY*
MRC Membrane Peptidase Research Group, Department of Biochemistry, University of Leeds, Leeds LS2 9JT, U.K.

The hydrolysis of the porcine 26-residue brain natriuretic peptide (BNP-26) and its counterpart human 28-residue atrial natriuretic peptide (α-hANP) by pig membrane preparations and purified membrane peptidases was studied. When the two peptides were incubated with choroid plexus membranes, the products being analysed by h.p.l.c., α-hANP was degraded twice as fast as BNP. The h.p.l.c. profiles of α-hANP hydrolysis, in short incubations with choroid plexus membranes, yielded αhANP′ as the main product, this having been previously shown to be the result of hydrolysis at the Cys[7]–Phe[8] bond. In short incubations this cleavage was inhibited 84% by 1 μM-phosphoramidon, a specific inhibitor of endopeptidase-24.11. BNP-26 was hydrolysed by choroid plexus membranes, kidney microvillar membranes and purified endopeptidase-24.11 in a manner that yielded identical h.p.l.c. profiles. In the presence of phosphoramidon, hydrolysis by the choroid plexus membranes was 94% inhibited. Captopril had no effect and, indeed, no hydrolysis of BNP-26 by peptidyl dipeptidase A (angiotensin-converting enzyme) was observed even after prolonged incubation with the purified enzyme. The stepwise hydrolysis of BNP-26 by endopeptidase-24.11 was investigated by sequencing the peptides produced during incubation. The initial product resulted from hydrolysis at Ser[14]–Leu[15], thereby opening the ring. This product (BNP′) was short-lived; further degradation involved hydrolysis at Ile[12]–Gly[13], Arg[8]–Leu[9], Gly[17]–Leu[18], Val[22]–Leu[23], Arg[11]–Ile[12] and Cys[4]–Phe[5]. Thus endopeptidase-24.11 is the principal enzyme in renal microvillar and choroid plexus membranes hydrolysing BNP-26 and α-hANP.

INTRODUCTION

The hydrolysis of the 28-amino-acid-residue human atrial natriuretic peptide (α-hANP) by porcine renal microvillar membranes is predominantly the result of an initial attack by endopeptidase-24.11 (Stephenson & Kenny, 1987*b*). The first bond to be hydrolysed by the purified enzyme is Cys[7]–Phe[8], thus opening the 17-residue ring and generating an inactive product, designated α-hANP′, which is eluted on h.p.l.c. with a shorter retention time than the intact peptide [for a review see Kenny & Stephenson (1988)]. This pattern of attack is common to both renal microvillar membranes and the purified endopeptidase and is inhibitable by phosphoramidon. Prolonged incubation with endopeptidase-24.11 further degrades α-hANP′ to smaller fragments, resulting from the hydrolysis at seven sites (Stephenson & Kenny, 1987*b*; Vanneste *et al.*, 1988).

Recently a peptide with pharmacological actions similar to those of α-hANP has been isolated from porcine brain and named 'brain natriuretic peptide' (BNP; Sudoh *et al.*, 1988*a*). The first form of BNP to be isolated was a 26-amino-acid-residue peptide (BNP-26) with considerable sequence similarity to α-hANP (Fig. 1). Another form, with an additional six residues at the *N*-terminus, BNP-32, is also present in brain (Sudoh *et al.*, 1988*b*) and a larger precursor form, of 12 kDa, has been found in pig heart (Minamino *et al.*, 1988). In pig brain BNP is present in 13-fold higher concentration than ANP (Ueda *et al.*, 1988). However, there appears to be some species variation, since radioimmunoassays have detected BNP in pig and dog brains but not in human, monkey or rat brain (Itoh *et al.*, 1989; Aburaya *et al.*, 1989*b*). Others, however, have been able to map BNP by immunohistochemistry in rat brain (Saper *et al.*, 1989). In peripheral tissues there are numerous reports of the presence of BNP peptides in cardiac atria, and cDNA clones encoding BNP precursors have been prepared from libraries of atrial tissue from several species (e.g. Aburaya *et al.*, 1989*a*; Kojima *et al.*, 1989; Seilhamer *et al.*, 1989). The peripheral actions of BNP and ANP are very similar (Hashiguchi *et al.*, 1988; Hirata *et al.*, 1988; Song *et al.*, 1988). When BNP was given by the intracerebroventricular route it had no effect on basal blood-pressure readings, but inhibited the pressor response of angiotensin II (Shirakami *et al.*, 1988). Although binding sites in tissues seem to be shared by both ANP and BNP (Oehlenschlager *et al.*, 1989), a receptor which preferentially binds BNP and is linked to a guanylate cyclase has recently been cloned from a human placental library (Chang *et al.*, 1989).

Little is known about the metabolism of BNP, but it has been shown to be degraded by dog kidney cortical membranes, and this hydrolysis was inhibited by phosphoramidon, showing endopeptidase-24.11 to be the enzyme initiating the attack (Vogt-Schaden *et al.*, 1989). Those authors also defined the points of hydrolysis by the membrane preparation as: Arg[8]–Leu[9], Ser[14]–Leu[15], Leu[15]–Ser[16], Ser[16]–Gly[17] and Gly[17]–Leu[18], the second being the principal point of attack generating a ring-opened product. Hydrolysis at Cys[4]–Phe[5], the initial attack on α-hANP, was not observed.

We have recently completed a biochemical and immunohistochemical survey of membrane peptidases in the choroid plexus (Bourne *et al.*, 1989). Most of the renal microvillar peptidases are present in porcine choroid plexus membrane preparations (only carboxypeptidase P, aminopeptidase P and membrane dipeptidase being undetected), but, with the exception of carboxypeptidase M, at much lower levels of activity. Endopeptidase-24.11 is uniquely located on the luminal surface of the choroid plexus epithelial cells, while aminopeptidase N and peptidyl dipeptidase A ('angiotensin-converting enzyme') have a dual location on epithelial and endothelial membranes. All three are also found on pial membranes, so that the

Abbreviations used: ANP, atrial natriuretic peptide; α-hANP, α-(human) atrial natriuretic peptide; BNP, brain natriuretic peptide (BNP-26, the porcine 26-residue peptide); iPr_2P-F, di-isopropyl fluorophosphate.

* To whom correspondence and reprint requests should be sent.

cerebrospinal fluid is enclosed by peptidase-rich membrane surfaces. Receptors for α-ANP are present in the choroid plexus (Bianchi *et al.*, 1986; Lynch *et al.*, 1986; McCarty & Plunkett, 1986), and intercerebroventricular injection of α-ANP decreases the production of cerebrospinal fluid by the cells of the choroid plexus (Steardo & Nathanson, 1987).

To date there has been only one report on the degradation of α-hANP by human choroid plexus membranes (Deschodt-Lanckman *et al.*, 1988). In that report the *C*-terminal tyrosine residue was iodinated and attention was focused on the hydrolysis of the -Ser[25]–Phe[26]- bond, which liberates the *C*-terminal fragment Phe-Arg-Tyr. Unlike kidney microvillar membranes, where this product is the result of endopeptidase-24.11 action (Stephenson & Kenny, 1987*b*), these authors found that in their crude choroid plexus membrane fraction only some 25% of this product was attributable to the action of endopeptidase-24.11, the rest being due to other metallo- and cysteine-endopeptidases.

In the present study we have reinvestigated the metabolism of α-hANP by the choroid plexus membranes previously characterized in this laboratory (Bourne *et al.*, 1989) and have compared this with the metabolism of porcine BNP-26 by membrane preparations and purified peptidases. We have found that endopeptidase-24.11 is the main enzyme responsible for the hydrolysis of both natriuretic factors.

EXPERIMENTAL

Enzymes, peptides and inhibitors

Endopeptidase-24.11 and peptidyl dipeptidase A were purified from pig kidney membranes (Gee *et al.*, 1983; Bull *et al.*, 1985). α-hANP was purchased from the Sigma Chemical Co., and BNP-26 was bought from the Peptide Institute (Scientific Marketing Associates, London N1 4RH, U.K.). Phosphoramidon was obtained from Protein Research Foundation, Osaka, Japan; amastatin and iPr_2P-F were from Sigma Chemical Co., and captopril was a gift from the Squibb Institute for Medical Research, Princeton, NJ, U.S.A.

Membrane preparations

These were prepared from pig, as previously described: renal microvillar membranes (Booth & Kenny, 1974) and choroid plexus membranes (Bourne *et al.*, 1989). For the choroid plexus membranes (pellet 2a) an additional washing step using mannitol/Tris homogenization buffer containing 250 mM-NaCl was introduced before resuspension.

Incubations with membranes and peptidases

The conditions were those described previously (Stephenson & Kenny, 1987*a*,*b*). For choroid plexus membranes the incubation mixture (volume 100 μl) contained 15 μg of protein.

α-hANP

SLRRSSCFGGRMDRIGAQSGLGCNSFRY

BNP-26

DSGCFGRRLDRIGSLSGLGCNVLRRY

Fig. 1. Sequences of α-hANP and BNP-26

Collection and analysis of peptide products by h.p.l.c.

This and all other methods were as described by Stephenson & Kenny (1987*a*,*b*), except that trifluoroacetic acid (0.05%) replaced phosphoric acid in some elutions. The amino acid analyses were performed by the Waters Picotag method after 24 h hydrolysis at 110 °C with 6 M-HCl containing 1% phenol, and the sequencing of the peptide products was performed by using an Applied Biosystems 477A protein sequencer.

RESULTS

Relative rates of hydrolysis of α-hANP and BNP by choroid plexus membranes

Both peptides were rapidly degraded by choroid plexus membranes (50 μM-peptide; membranes, 150 μg/ml, 37 °C, pH 7.4) in a pseudo first-order manner, which was linear up to about 50% hydrolysis. The approximate $t_{\frac{1}{2}}$ values under these conditions were 19 min for α-hANP and 40 min for BNP.

Products of hydrolysis of α-hANP incubation with choroid plexus

A study of the time course showed that some product peaks resolved by h.p.l.c. were transient (results not shown), but incubation for 25 min permitted adequate quantification of the main initial product peak (peak 4, Fig. 2*a*). In the presence of 1 μM-phosphoramidon (Fig. 2*b*) the area of this peak was reduced to 16%, whereas 10 μM-amastatin and 1 μM-captopril had no effect (results not shown). The areas of peaks 1, 2 and 3 were small, being less than 5% of that of peak 4, and were not accurately quantified, but they, too, appeared to be suppressed in the presence of phosphoramidon. The residual phosphoramidon-insensitive activity generating peak 4 was abolished by 1 mM-dithiothreitol, probably by its chelating action. The retention time of peak 4 is identical with that designated α-hANP′ (under the same conditions of elution) by Stephenson & Kenny (1987*b*) as the product of the initial cleavage by kidney microvillar membranes or purified endopeptidase-24.11. This product was later shown to be the result of hydrolysis at the Cys[7]–Phe[8] bond (Kenny & Stephenson, 1988). The amino acid analysis of the peptide in peak 4 corresponded to that of α-hANP′ and was therefore consistent with a ring-opening attack and may be identified as at the same bond.

Of the minor products after 25 min incubation, peak 2 was identified with the *C*-terminal fragment Phe-Arg-Tyr by co-elution with a synthetic marker peptide (Stephenson & Kenny, 1987*b*). In an extended incubation (120 min) the presence of 20 μM-amastatin enhanced peak 2 (Fig. 2*c*) under conditions where virtually no substrate remained. In a parallel incubation in the presence of phosphoramidon (Fig. 2*d*), no peak corresponding to Phe-Arg-Tyr was detected, but peak 4 (α-hANP′), attributable to the action of the phosphoramidon-insensitive enzyme(s), was enhanced compared with the 25-min incubations, 27% of the substrate being converted into α-hANP′.

Hydrolysis of BNP by choroid plexus, kidney membranes and by endopeptidase-24.11

Fig. 3 compares the h.p.l.c. elution profiles produced by the hydrolysis of BNP by choroid plexus, kidney microvillar membranes and purified endopeptidase-24.11, the conditions being selected to produce similar degrees of degradation of BNP. The three patterns are very similar. Seven product peaks can be discerned, of which peak 5 was shown to be the main initial product from a time course with choroid plexus membranes (results not shown). This product underwent further degradation on longer incubation, a process which was delayed about 30 min,

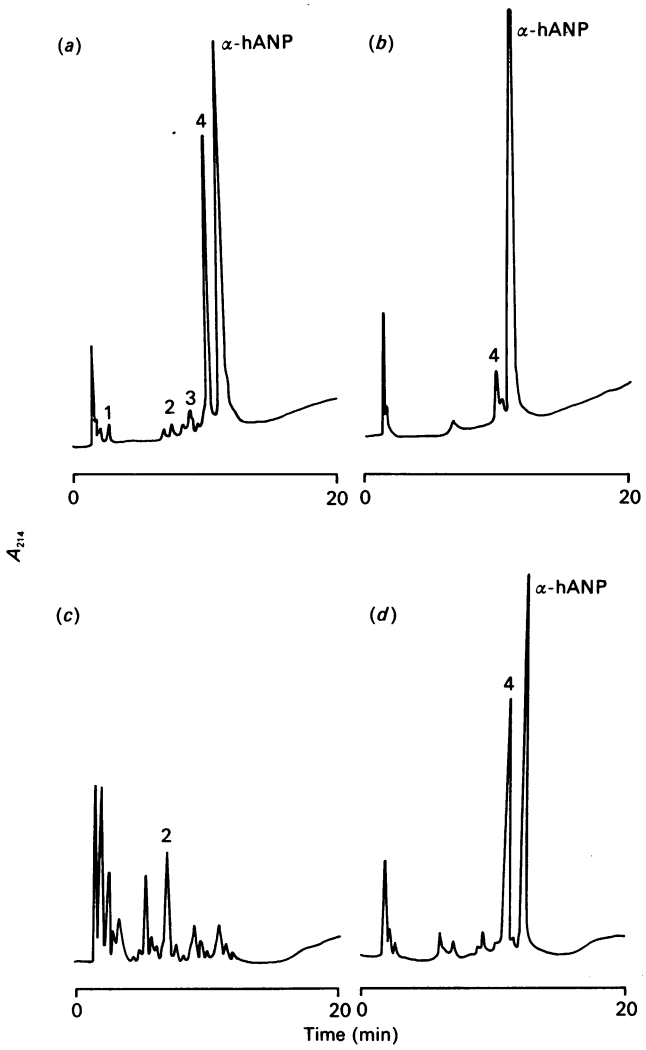

Fig. 2. Hydrolysis of α-hANP by choroid plexus membranes

The incubation (volume 100 μl) was terminated by heating to 100 °C for 5 min, the mixture centrifuged, and the supernatant analysed by h.p.l.c.; the elution buffer contained 0.08% phosphoric acid and a 4–45% (w/v)-acetonitrile gradient. (*a*) 25 min incubation; (*b*) as (*a*), but with 1 μM-phosphoramidon; (*c*) 120 min incubation with 20 μM-amastatin; (*d*) as (*c*), but with 1 μM-phosphoramidon. The two products to be identified were α-ANP′ (Peak 4) and the *C*-terminal fragment Phe-Arg-Tyr (FRY, Peak 2).

but not slowed, by 100 μM-amastatin. The similarity of pattern produced by the two types of membranes and by endopeptidase-24.11 is consistent with this enzyme being the principal peptidase in the attack. This point was supported, in a similar incubation with choroid plexus membranes, by the effects of inhibitors on the yields of the products (Table 1). In the presence of phosphoramidon, only 6% breakdown of BNP was observed, and the main product (peak 5) was decreased to 2% of the uninhibited control; the other relatively small peaks were 0–10% of the uninhibited value. Amastatin had an effect in increasing the yield of peak 1 and preventing the generation of peak 3, but both these peaks represented minor products. Captopril had no effect except that peak 4 was slightly enhanced. The ability of purified peptidyl dipeptidase A to hydrolyse BNP was investigated in a separate experiment in which 5 nmol of BNP was incubated with 100 ng of enzyme in the presence of 1 μM-phosphoramidon, 100 μM-amastatin and 100 μM-iPr_2P-F for 24 h at 37 °C. No decrease in the area of the BNP peak was observed and no product peaks were generated.

Bonds of BNP hydrolysed during incubation with endopeptidase-24.11

In a further experiment, BNP was incubated with purified

Table 1. Effect of peptidase inhibitors on the formation of the main products of BNP-26 incubated with choroid plexus membranes

The incubation conditions were those noted in the legend to Fig. 3. The peaks are numbered as in Fig. 3. The values for the experiment with phosphoramidon are means of duplicates for two experiments. Substrate hydrolysed (%) was 56 (no inhibitors), 6 (phosphoramidon), 50 (amastatin), 53 (captopril).

Peak no.	Retention time (min)	Peak area (arbitrary units)	Peptide remaining relative to that in absence of inhibitors (%)		
			Phosphoramidon (1 μM)	Amastatin (10 μM)	Captopril (1 μM)
1	7.34	168	0	250	100
2	9.48	360	0	95	110
3	10.11	291	10	0	88
4	10.98	874	2	110	160
5	11.72	1593	2	110	90
6	12.34	279	0	110	80
7	12.90	342	0	143	80

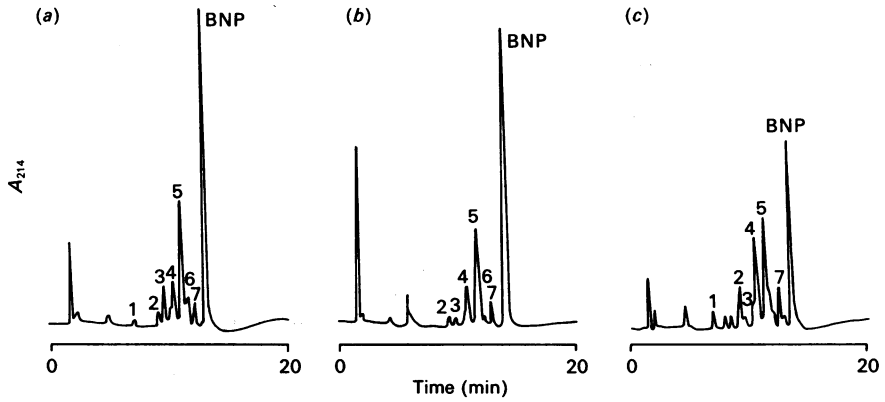

Fig. 3. Hydrolysis of BNP-26 by choroid plexus and kidney membranes and by endopeptidase-24.11

Incubations and analyses as in the legend to Fig. 2, except that the peptide was 50 μM-BNP. (*a*) 15 μg of choroid plexus membranes; 40 min incubation; (*b*) 2 μg of kidney microvillar membranes; 11 min incubation; (*c*) 70 ng of endopeptidase-24.11; 40 min incubation.

Table 2. Yields of the main products when BNP-26 was incubated with endopeptidase-24.11

The peaks from the four h.p.l.c. runs have been assigned Roman numerals to distinguish them from those in Table 1, because the elution system here contained trifluoroacetic in place of phosphoric acid. Note that peak I corresponds to peak 5 (being BNP′; the initial product of hydrolysis at Ser^{14}–Leu^{15}), peak II corresponds to peak 4, and peak III corresponds to peak 2. For the 48 h incubation the inhibitors, 10 μM-amastatin and 100 μM-iPr_2P-F were included. In this experiment all the incubation mixtures (volume 100 μl) contained 70 ng of purified endopeptidase-24.11 and 50 μM-BNP (i.e. 5 nmol). The yields were calculated from the sequences as previously described (Stephenson & Kenny, 1987*a*).

Peak	Retention time (min)	Identity of fragment	Time (h)...	Yield (nmol) during incubation 0.7	6	24	48
I	14.28	1–14, 15–26		0.6	—	—	—
II	13.36	1–12, 15–26		0.4	0.5	—	—
III	12.32	1–8, 18–26		0.1	1.0	0.1	0.0
IV	8.40	23–26		—	0.9	2.8	4.6
V	11.34	1–8, 18–22		—	0.5	1.3	1.1
VI	4.76	9–11		—	3.5	5.1	5.0
VII	6.86	5–7		—	0.5	2.0	1.4
VIII	9.12	1–4, 18–22		—	0.6	2.0	3.3

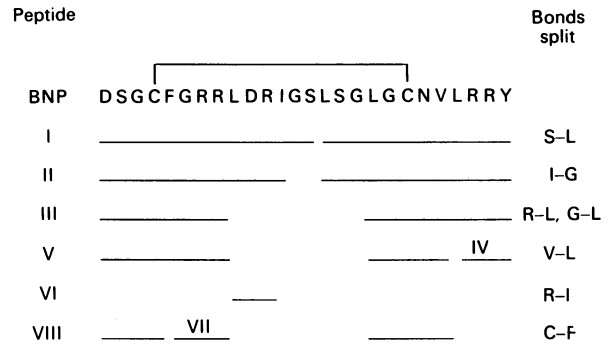

Fig. 4. Mode of hydrolysis of BNP-26 by endopeptidase-24.11

See the Experimental section and Table 2 for details. Lines indicate peptide fragments.

endopeptidase-24.11 for periods from 0.7 to 48 h (Table 2). The products were resolved by h.p.l.c and the main peaks collected for identification by sequencing. The initial product was the result of hydrolysis at Ser^{14}–Leu^{15}, this peak (referred to as I in Table 2) being identical with peak 5 in Table 1 and Fig. 3, in which experiments the elution system was slightly different. This product, which involves the opening of the ring, may, by analogy to α-hANP′, be called BNP′. BNP′ was short-lived, being undetected at 6 h. Other transient products were those defined as peaks II and III, which involved cleavage at Ile^{12}–Gly^{13}, Arg^{8}–Leu^{9} and Gly^{17}–Leu^{18}. The other products, peaks IV–VIII, either reached a plateau or increased throughout the incubation, there being stoichiometric yields (5 nmol) of products IV and VI. It may be noted that the inclusion of inhibitors of aminopeptidases and serine peptidases in the 48 h incubation had no effect on the products.

The pattern of attack is summarized in Fig. 4. The bonds observed to be cleaved in these incubations were Ser^{14}–Leu^{15}, Ile^{12}–Gly^{13}, Arg^{8}–Leu^{9}, Gly^{17}–Leu^{18}, Val^{22}–Leu^{23}, Arg^{11}–Ile^{12} and Cys^{4}–Phe^{5}. Some predicted fragments, Gly^{13}-Ser^{14} and Leu^{9}-Asp^{10}-Arg^{11}-Ile^{12}, were not seen in the elution profile.

DISCUSSION

Hydrolysis of α-hANP by choroid plexus

Although membrane peptidases are considerably less abundant in the choroid plexus membrane preparation compared with that from kidney microvilli (a difference for which compensation was made by increasing the membrane protein 7.5-fold in the incubation), the mode of attack was similar. The initial product, α-hANP′, resulted from the hydrolysis of the Cys^{7}–Phe^{8} bond mainly by the action of endopeptidase-24.11. However, at 25 min 16% of α-hANP′ was formed by a phosphoramidon-insensitive metallo-endopeptidase, and this activity was more significant at 120 min. The identity of the enzyme(s) responsible is not clear, but may relate to the phosphoramidon-insensitive activity seen with kidney microvillar membranes (Stephenson & Kenny, 1987*b*). However, the release of the *C*-terminal tripeptide by human choroid plexus membranes was reported by Deschodt-Lanckman *et al.* (1988) to be mainly the result of peptidase activity insensitive to phosphoramidon, whereas our study attributes nearly all of this cleavage to endopeptidase-24.11. The most probable explanation for this discrepancy lies in differences in the membrane preparations. In our experiments the preparation was a pellet, obtained after Mg^{2+} treatment, which had been subject to a washing step in 0.25 M-NaCl; in the other case it was a 15000 *g*/20 min crude membrane preparation that had not been washed free of cytosolic enzymes. However, it is also to be noted that we studied the degradation of 50 μM-α-hANP, whereas Deschodt-Lanckman *et al.* (1988) used a ^{125}I-labelled peptide at 30 pM.

Hydrolysis of BNP by choroid plexus membranes

When the two sequences are aligned (Fig. 1) with the half-cystine residues, there are 16 identical amino acids and several conservative substitutions. One might expect that the mode of attack by renal membranes would be similar for the two peptides. However, as reported by Vogt-Schaden *et al.* (1989), the Ser^{14}–Leu^{15} bond, rather than the Cys–Phe bond, was the preferred initial point of hydrolysis. Using a pure preparation of pig kidney endopeptidase-24.11 we have monitored the stepwise degradation of BNP and have confirmed that this bond is the first to be attacked. The subsequent steps involve the shortening of both portions of the opened ring by the removal of small fragments from the exposed ends. In this process each of the bonds cleaved conformed to the rule that the P_1' residue should be hydrophobic (see, e.g., Matsas *et al.*, 1984), with one exception, Ile–Gly, which was the second point of attack. The others generated Leu- or Phe- *N*-terminal fragments, the latter arising from the hydrolysis of the Cys^{4}–Phe^{5} bond. This was detected at 6 h and persisted throughout the 48 h incubation. Compared with the earlier report on BNP metabolism by dog renal

membranes (Vogt-Schaden *et al.*, 1989), we failed to observe the hydrolysis at Leu^{15}–Ser^{16} or Ser^{16}–Gly^{17}, but did find hydrolysis at Cys^{5}–Phe^{4} and Ile^{12}–Gly^{13}, which they did not observe. We confirm hydrolyses at Arg^{8}–Leu^{9}, Ser^{14}–Leu^{15} and Gly^{17}–Leu^{18}. These differences probably relate to their use of a crude membrane preparation, rather than a highly purified enzyme, as well as to the longer incubations used here to reveal all major sites of attack.

The h.p.l.c. profiles of BNP hydrolysis after short incubations with either choroid plexus membranes, renal microvillar membranes or purified endopeptidase-24.11 were identical. This, and the profound inhibitory effect of phosphoramidon, demonstrates that, in both types of membrane, endopeptidase-24.11 initiates the attack.

We thank Dr. J. N. Keen for the amino acid analysis, both Dr. Keen and Dr. M. J. Geisow for sequencing of the products of hydrolysis of BNP, and the Wellcome Trust for financial support.

REFERENCES

Aburaya, M., Hino, J., Minamino, N., Kangawa, K. & Matsuo, H. (1989*a*) Biochem. Biophys. Res. Commun. **163**, 226–232

Aburaya, M., Minamino, N., Hino, J., Kangawa, K. & Matsuo, H. (1989*b*) Biochem. Biophys Res. Commun. **165**, 880–887

Bianchi, C., Gutkowska, J., Ballak, M., Thibault, G., Garcia, R., Genest, J. & Cantin, M. (1986) Neuroendocrinology **44**, 365–372

Booth, A. G. & Kenny, A. J. (1974) Biochem. J. **142**, 575–581

Bourne, A., Barnes, K., Taylor, B. A., Turner, A. J. & Kenny, A. J. (1989) Biochem. J. **259**, 69–80

Bull, H. G., Thornberry, N. A. & Cordes, E. H. (1985) J. Biol. Chem. **260**, 2963–2972

Chang, M., Lowe, D. G., Lewis, M., Hellmiss, R., Chen, E. & Goeddel, D. V. (1989) Nature (London) **341**, 68–72

Deschodt-Lanckman, M., Vanneste, Y. & Michaux, F. (1988) Neurochem. Int. **12**, 367–373

Gee, N. S., Matsas, R. & Kenny, A. J. (1983) Biochem. J. **214**, 377–386

Hashiguchi, T., Higuchi, K., Ohashi, M., Minamino, N., Kangawa, K., Matsuo, H. & Nawata, H. (1988) FEBS Lett. **236**, 455–461

Hirata, Y., Shichiri, M., Emori, T., Marumo, F., Kangawa, K. & Matsuo, H. (1988) FEBS Lett. **238**, 415–418

Itoh, H., Nakao, K., Saito, Y., Yamada, T., Shirakami, G., Mukoyama, M., Arai, H., Hosoda, K., Suga, S., Minamino, N., Kangawa, K., Matsuo, H. & Imura, H. (1989) Biochem. Biophys. Res. Commun. **158**, 120–128

Kenny, A. J. & Stephenson, S. L. (1988) FEBS Lett. **232**, 1–8

Kojima, M., Minamino, N., Kangawa, K. & Matsuo, H. (1989) Biochem. Biophys. Res. Commun. **159**, 1420–1426

Lynch, D. R., Braas, K. M. & Snyder, S. H. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 3357–3361

McCarty, R. & Plunkett, L. M. (1986) Neurochem. Int. **9**, 177–183

Matsas, R., Kenny, A. J. and Turner, A. J. (1984) Biochem. J. **223**, 433–440

Minamino, N., Aburaya, M., Ueda, S., Kangawa, K. & Matsuo, H. (1988) Biochem. Biophys. Res. Commun. **155**, 740–746

Oehlenschlager, W. F., Baron, D. A., Schomer, H. & Currie, M. G. (1989) Eur. J. Pharmacol. **161**, 159–164

Saper, C. B., Hurley, K. M., Moga, M. M., Holmes, H. R., Adams, S. A., Leahy, K. M. & Needleman, P. (1989) Neurosci. Lett. **96**, 29–34

Seilhamer, J. J., Arfsten, A., Miller, J. A., Lundquist, P., Scarborough, R. M., Lewicki, J. A. & Porter, J. G. (1989) Biochem. Biophys. Res. Commun. **165**, 650–658

Shirakami, G., Nakao, K., Yamada, T., Itoh, H., Mori, K., Kangawa, K., Minamino, N., Matsuo, H. & Imura, H. (1988) Neurosci. Lett. **91**, 77–83

Song, D. L., Kohse, K. P. & Murad, F. (1988) FEBS Lett. **232**, 125–129

Steardo, L. & Nathanson, J. A. (1987) Science **235**, 470–473

Stephenson, S. L. & Kenny, A. J. (1987*a*) Biochem. J. **241**, 237–247

Stephenson, S. L. & Kenny, A. J. (1987*b*) Biochem. J. **243**, 183–187

Sudoh, T., Kangawa, K., Minamino, N. & Matsuo, H. (1988*a*) Nature (London) **332**, 78–81

Sudoh, T., Minamino, N., Kangawa, K. & Matsuo, H. (1988*b*) Biochem. Biophys. Res. Commun. **155**, 726–732

Ueda, S., Minamino, N., Sudoh, T., Kangawa, K. & Matsuo, H. (1988) Biochem. Biophys. Res. Commun. **155**, 733–739

Vanneste, Y., Michel, A., Dimaline, R., Najdovski, T. & Deschodt-Lanckman, M. (1988) Biochem. J. **253**, 531–537

Vogt-Schaden, M., Gagelmann, M., Hock, D., Herbst, F. & Forssmann, W. G. (1989) Biochem. Biophys. Res. Commun. **161**, 1177–1183

Received 2 April 1990/23 May 1990; accepted 30 May 1990