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# HORMONAL AND DRUG EFFECTS ON THE DEGRADATION OF HUMAN MYELIN BASIC PROTEIN PEPTIDE 43-88 BY ALKALINE PROTEOLYTIC ACTIVITY IN THE RAT KIDNEY

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**Abstract**—The microsomal brush-border fraction of rat renal tissue contains enzymatic activity, optimally active at pH 9, that is capable of degrading human myelin basic protein (BP) peptide 43-88. In the present study, this degradation and the effect on it of selected drugs and hormones were examined further. Of the substances tested, $10^{-2}$ M chloroquine and $10^{-5}$ M ACTH 1-24 were found to be the most effective inhibitors followed by $10^{-5}$ M ACTH 1-39; parathormone, glucagon and insulin were found to be inhibitors an order of magnitude weaker than ACTH 1-24. Hydrocortisone, dexamethasone, maleic acid and ACTH 4-10 were found to have minimal or no inhibitory effect on the peptide degrading activity. Gel filtration of the degradation products indicated that the rate of degradation of BP peptide 43-88 at pH 9 had been retarded by ACTH 1-24. These studies indicate that the clearance and catabolism of this peptide may be altered by available therapeutic agents.

Myelin basic protein (BP†), a highly cationic protein composed of 169 amino acid residues, constitutes approximately 30% of central nervous system (CNS) myelin proteins [1]. BP, which is capable of inducing inflammatory demyelination in animals [2], is released into the cerebrospinal fluid of humans at the time of damage to CNS myelin [3–5]. The route of the extraneural catabolism of BP or its peptides is unknown, but there is evidence that BP peptide 43-88, the major antigenic form of BP-like material in cerebrospinal fluid [6], is cleared and degraded by the kidney [7]. The subsequent metabolism and clearance of BP 43-88 are of interest because of the possible utility of measuring BP 43-88 in an accessible peripheral fluid compartment as a means of monitoring disease activity in multiple sclerosis [7]. In addition, in the BP molecule, which has multiple encephalitogenic determinants for different species of laboratory animals, BP peptide 43-88 contains the region of BP which can provoke experiment allergic encephalomyelitis in Lewis rats [8–10], rabbits [11, 12] and monkeys [13]. Hence, the catabolism of BP peptide 43-88 may have relevance to the potential autoimmunization with an encephalitogenic peptide having disease-inducing capacity.

On examination of rat renal homogenate, it was demonstrated that two enzymes were capable of degrading BP peptide 43-88 [12]. One of these is active at pH 5 with the features of cathepsin B, and the other, which greatly predominates, is a proteinase(s) with an optimum of pH 9 located in the microsomal brush border fraction [14]. The membrane-bound, alkaline metalloendopeptidase appears to be similar to a proteinase, termed meprin, demonstrated in kidney from selected strains of mice [15, 16]. The present investigation was undertaken to characterize further the size of the fragments formed in this degradation effected by the alkaline proteinase and to examine the influence of certain hormones and drugs on this process. The results indicate that physiological or pharmacological modification of the renal clearance and proteolysis is possible and may influence the immunological and biological consequences of circulating BP and BP peptides.

## MATERIALS AND METHODS

### *Materials*

Sodium iodoacetate, methylated bovine serum albumin (MBSA), ACTH 1-39 (A-6002), chloroquine diphosphate (C-6628), dexamethasone (D-1756), glucagon (G-4250), hydrocortisone (H-4001), indomethacin (I-7378), insulin (bovine, 1-5500), maleic acid (M-0375), D-mannitol (M-4125), parathyroid substance (TCA powder, P-0892), parathormone 1-34 (P-7149), phenylbutazone (P-8386) and 3-iodotyrosine (I-8250) were obtained from the Sigma Chemical Co. (St. Louis, MO). Iodohistidine was purchased from Calbiochem (LaJolla, CA) and crystalline bovine serum albumin (BSA) from Miles Laboratories (Elkhart, IN); ACTH 4-10 was obtained from Peninsula Laboratories (San Carlos,

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†Abbreviations: ACTH, adrenocorticotrophic hormone; BP, myelin basic protein; MBSA, methylated bovine serum albumin; P, peak from gel filtration; PTH, parathormone; TA-MBSA, Tris–acetate buffer containing MBSA; and TCA, trichloroacetic acid.

CA), and ACTH 1-24 was purchased from Organon, Inc. (West Orange, NJ). Molecular weight markers of ovalbumin and chymotrypsinogen A were obtained from Pharmacia (Piscataway, NJ) and carrier free Na $^{125}I$ from Amersham (Chicago, IL).

*Antigens and immunoassays*

Human BP peptide 43-88 was prepared by digestion of human BP with bovine brain cathepsin D followed by carboxymethyl-cellulose chromatography and gel filtration on Sephadex G-50 superfine [17]. This peptide was labeled with $^{125}I$ using lactoperoxidase-catalyzed iodination [14]. The immunoassay used for quantitating the degradation of radioiodinated BP peptide 43-88 was performed exactly as described previously [14] with the exception that the enzyme reaction mixture contained 200 μg/ml of crystalline bovine serum albumin (BSA) rather than MBSA and the sheep (number 104) antiserum to human BP peptide 43-88 was diluted 1:10,000. As described previously [14], the change to BSA was made, even though a small amount of non-specific adherence of radioligand to assay tubes occurred, because MBSA inhibited the peptide-degrading enzymatic activity. The greater dilution of sheep anti-BP peptide 43-88 showed no precipitation of radioligand in the absence of second antibody. The reaction of antibody with radioligand in the mixture was stated as percent soluble cpm, i.e. the change in immunoprecipitability (85% or more) of the undegraded radiolabeled BP peptide 43-88. The results were stated in this fashion for consistency with previously reported observations [14]. The inhibitor studies are reported as percent inhibition of degradation (i.e. solubilized cpm) over baseline uninhibited levels. Substances examined for their proteolytic effect were incorporated into the assay as previously described [14]. None was shown to alter the precipitation of radioligand by antibody.

*Tissue*

Rat tissue was obtained from male Sprague–Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 300–500 g. The tissue was homogenized and the 100,000 *g* pellet prepared [14]. Protein content of the fraction was determined by the Folin-phenol method [18].

*Gel filtration*

The products of human BP peptide 43-88 were analyzed by subjecting enzyme reaction mixtures to gel filtration though Sephadex G-50 superfine (1.5 × 90 cm) equilibrated at 4° with 0.2 M $NH_4HCO_3$. Five hundred microliters of incubation mixture was mixed with 500 μl of 0.2 M $NH_4HCO_3$ in a 12 × 75 polycarbonate tube, the radioactivity was determined, and the solution was applied to the column. Repeat determination of radioactivity of the emptied tube indicated over 99% of the cpm had been transferred. Radioactivity of the tubes containing column effluent was measured in a gamma counter. Columns were calibrated with ovalbumin, chymotrypsinogen A, Na $^{125}I$ and 3-iodotyrosine. The nonradioactive standards were monitored at 280 nm.

*Immunoprecipitability of column effluents*

Defined peaks from the gel filtration of undegraded or degraded radioligand were individually frozen, lyophilized twice, and subjected to immunoassay. The lyophilized residue was dissolved in 500 μl of 0.2 M Tris–acetate, pH 7.2, containing 0.2% (w/v) of MBSA (TA-MBSA). In duplicate, 200 μl of each dissolved specimen was mixed with 350 μl of TA-MBSA and 200 μl of a 1:10,000 dilution of S104 (anti-human BP peptide 43-88) [6] made in 0.2 M Tris–acetate, pH 7.2, containing a 1:200 dilution of normal sheep serum. After the mixture had stood at 4° for 16 hr, 200 μl of a 1:4 dilution of burro anti-sheep IgG was added and placed again at 4°. Six hours later the tubes were counted for radioactivity, the amount immunoprecipitated was calculated and the results were expressed, as described for the assay for degradation, as percent change in solubility of cpm [14].

*Hydrolysis of $^{125}I$-labeled peptides*

To determine procedures which enabled isolation and identification of the $^{125}I$-labeled amino acid residue in radiolabeled BP peptide 43-88 with a minimum loss of free $^{125}I$, standard 3-iodotyrosine (500 μg) was subjected to hydrolysis in constant boiling 6 N HCl, 6 N HCl plus 0.1% phenol and freshly prepared 0.2 N NaOH. The iodohistidine (500 μg) was subjected only to alkaline hydrolysis. Hydrolytic procedures were for 24 hr at 100° under one atmosphere of $N_2$ in a volume of 1 ml. The alkaline hydrolysis was conducted in borosilicate-free tubes (Corning No. 7380) using 0.2 ml of NaOH. After the alkaline hydrolytic procedure, the sample was diluted 10-fold with $H_2O$, chilled at 4° and the pH adjusted to 2.2 with 2 N HCl. Aliquots were taken for automatic amino acid analysis, and amino acids were separated with a four-buffer system on a single column as previously described [19].

*Effects on degradation*

Compounds tested were prepared fresh prior to each trial in solutions of borate buffer. There were no solubility problems with maleic acid, chloroquine or any of the ACTH preparations. Indomethacin and phenylbutazone were incompletely soluble even with the addition of 20% ethanol (v/v) in the preparatory mixture (2% in the final reaction mixture); after preliminary trials where no inhibition beyond that imposed by the ethanol itself was found, these two compounds were not tested further. Hydrocortisone and dexamethasone were slightly more soluble and were included in the study, with 1% ethanol (v/v) included in the final reaction mixture. This concentration of ethanol was found to cause a 3–5% increase in immunoprecipitability (which would indicate an apparent inhibition of degradation), and results with these drugs were corrected for this effect of ethanol. Insulin, glucagon, crude parathyroid hormone (PTH), and the isolated fragment PTH 1-34 were prepared as suspensions over the concentration range of $10^{-4}$ M through $10^{-7}$ M. In trials that included an inhibitor, the reaction volume was reduced from 450 to 400 μl, and 50 μl of the inhibitor was substituted in its stead.

Inhibitors were preincubated at 4° with enzyme mixtures for 30–45 min prior to the incubation at 37°. In a study performed to measure the effects of varying preincubation times from 0 to 60 min using $10^{-5}$ M ACTH 1-24 on the 100,000 *g* pellet, no differences in immunoprecipitability among the samples were observed. This was taken to indicate that preincubation, while not necessary in that instance, was not deleterious to the peptide or enzymes and could, therefore, be standardized.

## RESULTS

### *Identification of site of radioiodination of BP peptide* 43-88

Under the conditions used for amino acid analysis [19], lysine, histidine and arginine eluted as well-separated peaks near the termination of the run. Iodohistidine eluted before lysine while 3-iodotyrosine eluted immediately after arginine. The base hydrolysis resulted in approximately 50% loss of iodine from the standard 3-iodotyrosine and less than 20% from iodohistidine, the products being identified as either tyrosine or histidine respectively. With both of the acid hydrolytic procedures, complete loss of iodine from 3-iodotyrosine resulted, and free tyrosine was found. The radiolabled BP peptide 43-88 was therefore analyzed for $^{125}$I-labeled amino acids using base hydrolysis. The major peak of radioactivity co-eluted with standard 3-iodotyrosine while a second peak was not retained on the column and corresponded to free $^{125}$I. No radioactivity was detected in the elution fraction of iodohistidine. As with the control standard 3-iodotyrosine, approximately 50% of the $^{125}$I label was lost from the radioiodinated BP peptide 43-88 due to the alkaline hydrolytic procedure. Since the tyrosine residue at position 67 is the only tyrosine in human BP peptide 43-88 [12], these findings precisely localized the attachment of $^{125}$I in this peptide. There was no evidence for radioiodination of any of the five histidine residues [17] in human BP peptide 43-88. Thus, the peak of radioactivity detected following gel filtration would represent peptides containing the tyrosine at position 67.

### *Gel filtration: Calibration and immunoprecipitability of effluent*

On a calibrated column of Sephadex G-50 superfine, radioiodinated human BP peptide 43-88 eluted as a major peak at 59–65% (P62) of the gel bed volume with the two smallest peaks preceding it at 35–39% (P37) and 46–50% (P48) of the gel bed volume (Fig. 1). The relative heights of P37 and P48 varied among different preparations of human radiolabeled BP peptide 43-88. The first two peaks probably represent a dimer or polymer of BP peptide 43-88, but the possibility that a small amount of radiolabeled lactoperoxidase was present has not been excluded. The peak of radioactivity at 90–94% (P92) of the gel bed volume eluted at the same volume as free iodine and was interpreted to be free $^{125}$I not removed after radiolabeling. 3-Iodotyrosine eluted at 100–110% (P105) of the gel bed volume. Following immunoprecipitation with S104 antiserum, the solubilities of the fractions from the separation of undigested radioligand on the G-50 superfine column were 37% for P37, 0% for P48, 0% for P62, and 84% for P92 (Table 1).

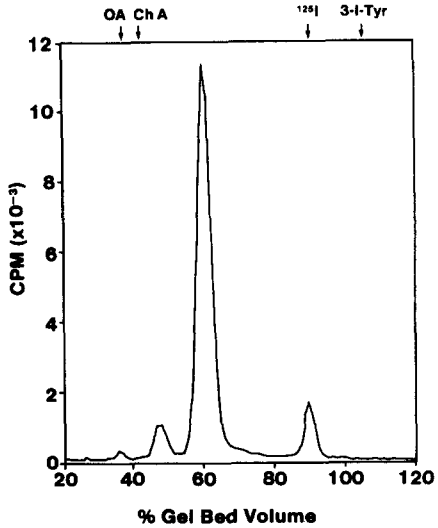


Fig. 1. Gel filtration, on Sephadex G-50 superfine (1.5 × 90 cm) in 0.2 M $NH_4HCO_3$, of radioiodinated BP peptide 43-88. Molecular weight markers (↓) used were ovalbumin (OA) (mol. wt = 45,000) and chymotrypsinogen A (ChA) (mol. wt = 25,000). Areas are also designated for elution of free $^{125}$I and 3-iodotyrosine (3-I-Tyr). Approximately 61,000 cpm were applied to the column.

Table 1. Post-immunoprecipitation solubility of column fractions of radioiodinated BP peptide 43-88 before and after degradation by renal microsomal brush border fraction (100,000 *g* pellet) at pH 9

| Column fraction | Solubility | Treatment of $^{125}$I-labeled BP peptide 43-88 |
|---|---|---|
| P37* | 37† | None (before degradation) |
| P48 | 0 | None (before degradation) |
| P62 | 0 | None (before degradation) |
| P77 | 43 | Renal 100,000 *g* pellet |
| P92 | 84 | None (before degradation) |
| | 84 | Renal 100,000 *g* pellet |
| P105 | 94, 87 | Renal 100,000 *g* pellet |

* Number indicates the percent of the gel bed volume of a Sephadex G-50 superfine column at which the peak (P) eluted.

† Number indicates percent solubility of cpm following immunoprecipitation by S104 anti-human BP peptide 43-88.

### *BP peptide* 43-88 *degradation by the* $10^5$ g *pellet of renal homogenate at pH* 9

On exposure of radiolabeled human BP peptide 43-88 at pH 9 to the $10^5$ *g* pellet of rat renal homogenate, there was also a rapid decrease in P62 as well as P48 followed by the appearance of a prominent, later eluting peak at 75–80% (P77) of the gel bed volume (Fig. 2A). P77 ultimately decreased with the concomitant appearance of numerous smaller peaks at 102–118% of the gel bed volume (Fig. 2, B–D). Simultaneous with the decrease of P62, there was a decrease or disappearance of P37 and P48. P92 broadened and became more prominent.

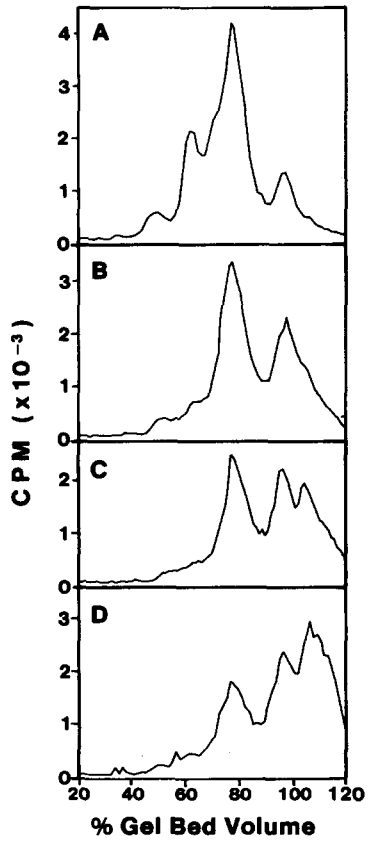


Fig. 2. Gel filtration, on Sephadex G-50 superfine (1.5 × 90 cm) in 0.2 M $NH_4HCO_3$, of radioiodinated human BP peptide 43-88 exposed at 37° to a solution (15 μg protein/ml) of the 100,000 *g* pellet of rat renal homogenate at pH 9 for 5 (A), 15 (B), 30 (C) and 60 (D) min. The increased solubility (loss of immunoprecipitability) of cpm was 14% for A, 31% for B, 47% for C, and 58% for D. Approximately 60,000 cpm were applied to each column.

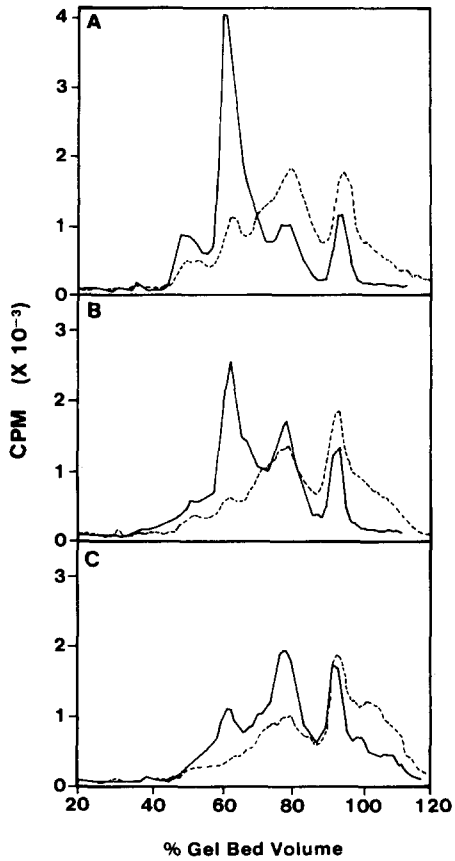


Fig. 3. Gel filtration on a 1.5 × 90 cm column of Sephadex G-50 superfine of $^{125}I$-labeled human BP peptide 43-88 following digestion by the 100,000 *g* pellet of rat renal enzyme homogenate at pH 9 for incubation periods of (A) 15 min, (B) 30 min and (C) 60 min, at 37° in the presence (solid line) and absence (dashed line) $10^{-5}$ M ACTH 1-24. See Fig. 1 for the elution profile of undigested human BP peptide 43-88 and the calibration points.

Table 2. Percent inhibition over baseline levels (± S.E.M.) of the degradation of BP 43-88 by the rat renal brush border enzymes, pH 9, by specified concentrations of ACTH and ACTH fragments*

| Peptide | % Inhibition | | | |
|---|---|---|---|---|
| | $10^{-4}$ M | $10^{-5}$ M | $10^{-6}$ M | $10^{-7}$ M |
| ACTH 1-39 | 91.0†‡ (2.8)§ | 55.4† (7.9) | 10.0 (5.0) | 14.0 (8.2) |
| ACTH 1-24 | ND‖ | 84.0†¶ (8.3) | 22.6 (8.4) | 7.7 (6.8) |
| ACTH 4-10 | 23.0 (9.0) | 12.2 (7.4) | 2.8 (4.5) | 0.0 (0.0) |

* Unless otherwise noted, the number of observations for each entry was between 3 and 6.
† $P < 0.01$ by Kruskal–Wallis procedures.
‡ Two observations.
§ Numbers in parentheses are the S.E.M.
‖ Not done.
¶ Fifteen observations.

Table 3. Percent inhibition over baseline levels (± S.E.M.) of the degradation of BP peptide 43-88 by the rat renal brush border enzymes, pH 9, by specified concentrations of selected peptide hormones*

| Hormone | % Inhibition | | | | | | |
|---|---|---|---|---|---|---|---|
| | $10^{-4}$ M | $5 \times 10^{-5}$ M | $2 \times 10^{-5}$ M | $10^{-5}$ M | $5 \times 10^{-6}$ M | $10^{-6}$ M | $10^{-7}$ M |
| Glucagon | 67.0† (5.2)‡ | ND§ | 44.3† (2.3) | 24.0 (6.0) | ND | 15.0 (3.0) | 10.0 (3.5) |
| Insulin | 72.7† (8.0) | 46.7† (3.5) | ND | 14.7 (2.2) | ND | 10.2 (5.2) | 8.8 (5.9) |
| PTH (Crude) | 74.0† (3.0) | ND | 49.0† (1.7) | 19.0 (4.6) | 22.0 (9.5) | 6.8 (6.7) | 6.7 (7.3) |
| PTH 1-34 | ND | ND | ND | 17.0 (1.7) | ND | 14.0 (1.7) | 14.0 (3.5) |

* The number of observations for each entry was between 3 and 6.
† $P < 0.01$ by Kruskal–Wallis procedures.
‡ Numbers in parentheses are the S.E.M.
§ Not done.

*Inhibition of degradation of BP peptide* 43-88

*ACTH and ACTH peptides.* Studies with ACTH and ACTH peptides were conducted over a concentration range of $10^{-5}$ M through $10^{-9}$ M, similar to that chosen by others [20, 21]. Initial experiments indicated no effective inhibition by ACTH 1-39 at concentrations less than $10^{-7}$ M; this was therefore used as a lower limit in further studies. The effectiveness of different ACTH peptides on the pH 9 (100,000 *g* pellet) enzyme system are presented in Table 2; the number of observations made and the standard error (S.E.M.) of each set of observations are also included. Except as noted, each entry is the mean of from three to six independent observations. Results are presented as percent inhibition over baseline controls without added inhibitor. As may be seen in Table 2, ACTH 1-24 was the most effective inhibitor at $10^{-5}$ M, reaching 84% inhibition at $10^{-5}$ M, with some inhibitor effect still detected at a concentration of $10^{-6}$ M. ACTH 1-39 was effective over a similar range of concentrations on a molar basis but was not as potent as ACTH 1-24. Using the Kruskal–Wallis non-parametric procedure for k-independent samples [22], ACTH 1-39 and ACTH 1-24 at concentrations of $10^{-5}$ M and greater were significantly inhibitory over baseline levels ($P < 0.01$); at no lower concentration was significance ($P > 0.05$) achieved and at no concentration tested was ACTH 4-10 a significant inhibitor ($P > 0.05$).

The ACTH 1-24 used was obtained mixed with D-mannitol at a ratio of 1:40. Control studies of D-mannitol alone, but in the same concentrations achieved with ACTH 1-24, were performed and disclosed no inhibitory effect of D-mannitol. Studies conducted over a pH range of 4–12 in the presence and absence of $10^{-5}$ M ACTH demonstrated that ACTH neither shifted the pH optimum of the enzyme system nor altered the pH of the incubation mixture. This control confirmed that inhibition, not alteration of the pH from the optimal pH range for the degradation [14], had occurred. The time course of the degradation of BP peptide 43-88 at pH 9 with and without ACTH 1-24 was also studied. After 0, 15, 30, and 60 min, the incubation mixtures were subjected to gel filtration on Sephadex G-50 superfine, and the fractions collected were analyzed for radioactivity. In the absence of ACTH, the immunoprecipitability of BP peptide 43-88 was 87% before the incubation and 67, 58 and 36% at 15, 30 and 60 min respectively. In the presence of $10^{-5}$ M ACTH 1-24, these levels were 90, 82 and 73% respectively.

The elution profile of radioactivity from Sephadex G-50 superfine of each of these timed incubation mixtures paralleled the changes in immunoprecipitability. Alteration in the elution profile occurred in the absence and presence of ACTH 1-24 but was appreciably slower when the ACTH peptide was present (Fig. 3). BP peptide 43-88 was degraded to progressively smaller radiolabeled peptides, some eluting beyond the gel bed volume for Na $^{125}$I but in the position of 3-iodotyrosine (Fig. 1). There was no evidence of a difference in size of radiolabeled peptides formed in the presence or absence of ACTH 1-24.

*Parathyroid hormone, insulin and glucagon.* To ascertain whether any protein or peptide over a certain length might function non-specifically as an inhibitor of the 100,000 *g* pellet enzyme system, other hormones were tested for an inhibitory effect. Glucagon, insulin, crude parathormone (PTH) and parahormone peptide 1-34 (PTH 1-34) were examined over a concentration range of $10^{-4}$ M to $10^{-7}$ M. The results are presented in Table 3, with entries as percent inhibition over baseline control levels as before. Once again employing the Kruskal–Wallis techniques, statistical significance ($P < 0.05$) was not achieved until $10^{-5}$ M concentrations were exceeded. Glucagon, insulin and PTH achieved statistically significant effects ($P < 0.01$) at $2 \times 10^{-5}$ M (44.3%), $5 \times 10^{-5}$ M (46.7%) and $2 \times 10^{-5}$ M (49.0%) respectively. (The few intermediate values between $10^{-6}$ M and $10^{-5}$ M, and between $10^{-5}$ M and $10^{-4}$ M, were determined during preliminary trials. They are included here as evidence of consistently progressive inhibition with increasing concentration of peptide hormone.)

*Selected steroids and drugs.* Hydrocortisone, dexamethasone, chloroquine, and maleic acid were tested at various concentrations in the pH 9 system. Hydrocortisone and dexamethasone were tested at concentrations from $10^{-8}$ M to $10^{-3}$ M [23]; maleic acid [24] and chloroquine [25] were evaluated over the range $10^{-4}$ M to $10^{-2}$ M. At pH 9, only chloroquine was found to be an effective inhibitor, and only then in concentrations equal to or greater than $10^{-3}$ M. With $10^{-2}$ M chloroquine, inhibition reached 92.2% over baseline (S.E.M. = 9.3, N = 6, $P < 0.01$); at $10^{-3}$ M, inhibition dropped to 101.8% (S.E.M. = 5.7, N = 4, $P > 0.05$). (Although chloroquine diphosphate itself is a strong acid, the reaction mixture was titrated to pH 9.0 with 2 N NaOH prior to the trial, removing pH shift as an explanation.)

## DISCUSSION

If BP plays a role in autoimmune demyelination in humans, the biological and immunological effects of endogenously released BP or BP peptides are likely to be influenced by the concentration and type of peptide present. The identification of the kidney as a major site for the clearance and degradation of BP peptide 43-88 [7, 14] indicates that factors affecting the renal handling of BP peptides will, in turn, modify the effects of BP peptides on the immune and other systems. Hence, physiological and pharmacological substances may exert an effect on the host response to BP peptides through an effect on the clearance and degradation of BP peptides rather than by modifying the immune response itself. The present investigation was undertaken as an initial exploration of the inhibitory effects of selected substances on the degradation of BP peptide 43-88 by the major renal proteinase in the rat degrading this peptide. ACTH was tested because of its known influence on protein degradation [21, 26, 27] and because of its clinical utilization in the treatment of a variety of diseases including multiple sclerosis [28] and other neurological disorders [29]. Other compounds tested were chosen because of their effects on inflammation or renal function. Some of the materials tested were capable of slowing the degradative processes. It is conceivable that other materials may be capable of enhancing the degradation.

The effect of ACTH and its peptides in diminishing degradation of peptides is well established. ACTH inhibits the *in vitro* degradation of glucacon by renal proteinases [26, 27] and increases the *in vivo* half-life of adrenal proteins [30]. Studies of adrenal tumor cells have revealed that ACTH 1-39, ACTH 1-24 and nitro phenylsulphenyl-ACTH 1-24 are effective inhibitors of protein degradation [20] but that ACTH 1-10, ACTH 4-10 and ACTH 18-39 are not effective [21]. ACTH 1-24, ACTH 1-10 and other neuropeptides inhibit the degradation of met-enkephalin by an aminopeptidase present in rat brain microsomes [31].

Results of the present study indicate that the enzyme(s) in the 100,000 *g* pellet which is active at pH 9 in degrading human BP peptide 43-88 can be inhibited by ACTH peptides 1-39 and 1-24 while ACTH peptide 4-10 had minimal effect. Analysis by gel filtration of the degradation products produced in the presence or absence of ACTH 1-24 demonstrated that ACTH 1-24 inhibited the rate of breakdown of BP 43-88 but changed very little the size of the breakdown products themselves. The inhibitory potential of crude PTH, PTH 1-34, glucagon, and insulin was less than half that of ACTH 1-39 and less than one-third that of ACTH 1-24 at $10^{-5}$ M. This suggests two possible inhibitory effects for ACTH in this enzyme system: first, a non-specific role shared by several peptide hormones and second, a peptide sequence-specific role. Morel *et al.* [21] have argued that the lack of inhibitory function of the peptides ACTH 1-10, 4-10 and 18-39 on the degradation rate of Y-1 adrenal cell protein is due to sequence specificity unique to ACTH fragments including peptide 1-24. The results of the present study suggest that the peptide region of 1-24 (possibly as little as 11-24) is the active portion of ACTH inhibiting the degradation.

Hydrocortisone, dexamethasone, maleic acid, and chloroquine were also tested on the proteolytic activity in the 100,000 *g* pellet fractions. Of these, only chloroquine at the high concentration of $10^{-2}$ M was effective. Similar concentrations of chloroquine have been shown to inhibit hepatocyte protein degradation [32] and lysosomal cathepsin $B_1$ [25]. Although seemingly extreme, $10^{-2}$ M concentrations may be achievable *in vivo* in specific compartments. Rat fibroblasts are able to concentrate an external $10^{-4}$ M chloroquine concentration to $2 \times 10^{-2}$ M, with the lysosomal concentration reaching $10^{-1}$ M [25]. Given that peak therapeutic plasma concentrations generally reach $10^{-6}$ M [33], it may be seen that such concentrations as $10^{-3}$–$10^{-2}$ M chloroquine might, in theory, be reached in certain subcellular fractions. In contrast to chloroquine, maleic acid may exert a potent toxic effect on renal proximal tubule epithelium [34], but the mechanism for this deleterious effect remains uncertain [22]. This study provided no evidence that *in vitro* maleic acid altered the degrading activity of the rat microsomal brush border fraction for BP peptide 43-88.

This work does not address the problem of mechanism of the inhibitory process or processes but does demonstrate that the degradation of BP peptide 43-88 by rat renal enzymes can be inhibited by endogenously produced substances and currently available by therapeutic agents. These findings indicate that the clearance and catabolism of this potentially autoantigenic peptide can be altered by physiological and pharmacological means.

Information on the catabolic events of BP peptide 43-88 was also sought in this investigation for two additional reasons. First, although antigenic material in cerebrospinal fluid cross-reacting with BP peptide 43-88 serves as a useful indicator for CNS myelin damage in humans [4–6], the clinical feasibility of using BP immunoreactivity as an index of CNS myelin damage necessitates its detection in a more accessible body fluid such as blood or urine. Second, since small fragements of BP may be capable of causing experimental allergic encephalomyelitis [10, 35] and possibly other biological events [36], identification of the small BP fragments formed may yield information concerning the role of this potential

autoantigen in humans and permit the recognition of abnormalities of its extraneural metabolism. Although the reliance on the profile of radiolabeled peptide fragments would indicate the smallest possible number of generated fragments and underestimate the variety and complexity of generated fragments, the present findings show that an endopeptidase is involved in this degradative process. This demonstrates that large fragments, some containing the tyrosine at position 67, of BP peptide 43-88 appear during the degradation of the peptide *in vitro*. If similar events occur *in vivo*, BP fragments of sufficient size should exist for identification and measurement in urine and, if absorbed from the renal tubule, in blood.

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