ANGIOEDEMA INDUCED BY A PEPTIDE DERIVED
FROM COMPLEMENT COMPONENT C2

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Several lines of experimental evidence have suggested that one or more peptides that can enhance vascular permeability may be released from the second component of human complement, C2 (1–5). This possibility is important to understanding the molecular mechanisms that lead to bouts of circumscribed edema to which persons with hereditary angioneurotic edema (HANE)[1] are susceptible. Persons with this disorder have defects in the biosynthesis of C1 inhibitor, which are inherited as autosomal dominant traits and are markedly deficient in serum inhibitory activity directed against the activated first component of complement, C1 (6, 7). An acquired form of this disease, which occurs as a result of C1 inhibitor depletion, has also been reported (8–11).

The mediators of the enhanced vascular permeability that leads to edema formation when C1 inhibitor is deficient are not known. C1 activity is strictly controlled by C1 inhibitor (12), which binds covalently to the C1 complex (13–16). Since C1 inhibitor is the only identified inhibitor of C1r and C1s, the absence or depletion of this plasma component results in a loss of regulation of the early steps of the classical complement pathway. It has been shown in patients with HANE that C2 and C4 levels are low during and after an attack (1). The present studies are a direct approach to determining if C2 fragments, which can be released by C1 and other plasma proteases inhibited by C1 inhibitor, contribute to enhanced vascular permeability and subsequent bouts of angioedema. We have found that a synthetic peptide of 25 residues identical in sequence to residues 199–223 of the COOH terminus of C2b has such a property.

Materials and Methods

Bradykinin. Commercially available synthetic bradykinin (Bacham Laboratory, Torrance, CA) eluted as a single sharp peak upon HPLC analysis using application of a 0.0–70.0%

(vol/vol) acetonitrile gradient in 0.1% (vol/vol) TFA onto a reverse-phase column (Brownlee C18; Rainin Instrument Co., Inc., Woburn, MA) and was then used without further purification. As a standard for biological assays, bradykinin at 1 mg/ml was made in 0.1% acetic acid and the exact concentration was determined by compositional analysis of the solution.

Enzymes.   Human C1s, in the enzymatically active form and free of contaminating C1r and plasmin enzymatic activities, was a gift from Dr. David Bing (Center for Blood Research, Boston, MA). The concentration of C1s is given in micrograms as determined using an extinction coefficient at 280 nm of 0.94 ml/(mg cm).

Human plasminogen was either a gift from Dr. Eileen Remold-O'Donnell (Center for Blood Research) or, for some experiments, purified using a published procedure (17) involving plasma adsorption to lysine-Sepharose and subsequent elution of plasminogen with ε-aminocaproic acid (Sigma Chemical Co., St. Louis, MO). Immediately before use the plasminogen was converted to plasmin by incubation with purified streptokinase as previously described (18). The conversion from plasminogen to plasmin was always confirmed by SDS-PAGE (19) under reducing conditions. In the presence of 3% (vol/vol) β-ME, plasminogen migrated as a single band of 100,000 M_r and plasmin migrated as two bands of 70,000 and 35,000 M_r.

Human C2.   C2 was purifed according to Thielens et al. (20) with certain modifications and additions. The changes are as follows: fresh frozen plasma (Knickerbocker Biological, New York, NY) was thawed in the presence of 5 mM benzamidine HCl (Sigma Chemical Co.) and 10^{-4} M diisopropylfluorophosphate (DFP). The partially thawed "slush" of plasma was then dialyzed against sodium phosphate/EDTA with 5 mM benzamidine and 10^{-5} M DFP added. No β-ME was added to the ammonium sulfate solutions, and the precipitate formed at 75% saturation was centrifuged for 60 min. In preparation for CM-Sepharose chromatography, the precipitiate was dissolved in 20 mM sodium phosphate, 2 mM EDTA, pH 7.0, to which 5 mM benzamidine and 10^{-4} M DFP had been added and then dialyzed against this buffer. Just before CM-Sepharose chromatography, the pH of the dialyzed pool was acidified to pH 6.0 with 1 M HCl. A CM-Sepharose column with a resin volume equivalent to three times the C2 pool volume and which had been equilibrated in the sodium phosphate/EDTA, pH 6.0, buffer was used. The final yield for this procedure was 35–40%[2].

C2 Digestion and Peptide Isolation.   Purified C2 at 1–2 mg/ml was dialyzed into 50 mM NH_4HCO_3 and then the dialyzed protein was digested with C1s (1:100, wt/wt) and plasmin (1:20, wt/wt) for 1 h at 37°C. After incubation, the protein solution was either lyophilized in preparation for HPLC fractionation or analyzed by SDS-PAGE.

The C2b-derived peptides were isolated by a series of two HPLC steps: chromatography of the digest mixture on an Aquapore reverse-phase C8 column, followed by chromatography of the peptide fraction on a Brownlee reverse phase C18 column. Both HPLC columns were purchased from Rainin Instruments. The lyophilized digest mixture was dissolved in 0.1% TFA (vol/vol) and applied to the C8 column, which was equilibrated in 0.1% TFA, 5% acetonitrile. The proteolytic fragments were then eluted using a linear acetonitrile gradient of 5–70% (vol/vol) in 0.1% TFA over 30 min. The fractions containing peptides that eluted early were then rechromatographed on the C18 column, using the same chromatographic conditions that were used for the C8 column.

Synthetic Peptides.   Directly after cleavage from the Merrifield resin with HF, the synthetic peptides were lyophilized 8–10 times from water to remove volatile components. The peptides were then fractionated on a P2 column (5 ml; Bio-Rad Laboratories, Richmond, CA), equilibrated in 0.2 M NH_4HCO_3, pH 8.2, then lyophilized, lyophilized again from H_2O (three times), and finally brought up in sterile 0.9% saline for testing. Amino acid analysis of P2-fractionated synthetic peptides of 19 amino acids or shorter in length were nearly integral, indicating peptides of substantial purity. When analyzed by reverse-phase HPLC, these same peptides showed a major peak, which accounted for >90% the sample peptide mass; by amino acid analysis, this peak was full-length peptide. The amino acid analyses for the P2 purified synthetic peptides used in this study are shown in Table I.

Results from amino acid analysis on the P2-purified C2b 199–223 peptide did not corre-

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[2] Virtually all the loss of C2 occurs in the two chromatographic steps; a typical purification table that includes the yield of C2 at each step in the procedure has been previously published (35).

TABLE I

Amino Acid Compositions of the Synthetic Peptides of C2b

Peptide	Amino acid compositions*												
	Asp	Thr	Glu	Pro	Lys	Ser	Leu	Gly	Arg	Ala	Met	Phe	His
C2b 212-216	0.88	1.02	1.08	0.98	1.03								
C2b 219-223			0.96			0.85	0.94	1.10	1.15				
C2b 217-223		0.86	0.97		0.87	0.95	0.97	1.16	1.22				
C2b 214-223		1.92	2.27		2.04	0.74	1.08	0.84	1.10				
C2b 207-223	1.05	3.11	2.33	1.29	2.15	0.79	1.85	1.71	1.11	0.95	0.66		
C2b 199-223	1.71	3.54	3.59	1.81	2.00	3.11	1.96	10.12	1.45	2.06	0.43	0.44	0.91

* Mole of amino acid per mole of peptide.

late exactly with the theoretical amino acid composition and HPLC analysis of this peptide showed several peaks. From amino acid analysis of the peptide-containing HPLC fractions (data not shown), this sample was found to be 25% (wt/wt) full-length peptide. Calculations of biological activity reflect this ratio.

NH_2-terminal Amino Acid Sequence Analysis.   Automated Edman degradation was performed using 25% trifluoroacetic acid at 50°C for 27 min with a sequencer updated with microprocessor control and automated conversion (Beckman Instruments, Inc., Fullerton, CA). A 0.1 M Quadrol program was used. Phenylthiohydantoin derivatives were identified by HPLC using a Zorbax PTH column developed isocratically with 6 mM phosphoric acid, pH 3.15, acetonitrile, and tetrahydrofuran, (66:19:15).

Peptide Synthesis.   Synthetic peptides were made by solid-phase synthetic techniques as developed by Merrifield and colleagues (21, 22).

SDS-PAGE.   PAGE under denaturing and reducing conditions was performed as described (19), with one exception; to separate C2 and factor B, an acrylamide/bis acrylamide ratio of 30:0.016 was used.

C2 Functional Activity.   C2 was assayed for functional activity using a hemolytic assay described by Rapp and Borsos (23). Sheep erthrocytes sensitized with anti-Forsmann antibody and bound C1 (guinea pig) and C4 (human) on the surface were used as a source of early complement components and rat serum (1:3 dilution into veronal buffered saline with 10 mM EDTA, pH 7.35, and gelatin [0.1%, wt vol] [GVB-EDTA]) was used as a source of C3-9. The C2 titer of the partially purified C2 was compared with a C2 titer determined for normal human serum to estimate the concentration of functional C2. In addition, C2 was found to be free of plasma kallikrein as judged by the inability of C2 fractions to reconstitute Fletcher factor-deficient plasma (George King Biomedical, Inc., Overland Park, KS) as described (24). The purified C2 was also found to be free of kinin precursors as demonstrated by the lack of contractile activity on estrous rat uterus after incubation with human urinary kallikrein at 37°C for 10 min.

Induction of Rat Uterus Contraction.   The ability of each peptide to induce contraction of a rat estrous uterus was used to test the biological activity of these peptides (25). A portion (1-2 cm) of excised rat uterus was hung in an aerated bath (5 ml) of deJalon's buffer, and connected to a strain gauge transducer (Adaps, Inc., Dedham, MA). The uterus was standardized with a solution of bradykinin (0.1-1 pmol) in water, the concentration of which was determined by amino acid analysis. The synthetic C2b peptides in 50 mM acetic acid were then tested. Buffers of PBS or normal saline were also used for solubilization of the peptides. A standard curve for bradykinin was determined three or four times per day and doses of bradykinin that induced 20% and 70% maximal contraction were given just before and immediately after a dose of synthetic peptide. The solution concentration for each synthetic peptide was standardized by compositional analysis. For biological measurements of the C2b fragments that had been generated by C1s and plasmin, fractions were collected in plastic tubes, lyophilized, and dissolved in 50 mM acetic acid (200 µl), and assayed as described.

Skin Vascular Permeability.   Adult guinea pigs (500-800 g) were assayed for skin vasoperme-

ability by Evans Blue extravasation. Evans Blue (0.1% in 0.15 M NaCl, 0.2 ml/100 g body weight) was injected intravenously into the foreleg. The abdominal area of the guinea pig was then shaved and the peptides in 0.1 ml PBS were injected intradermally. The extent of vascular permeability enhancement was assessed as described previously (5).

To quantify the enhancement of human skin vascular permeability, the peptides in 0.1 ml sterile saline were injected intradermally into the forearm of normal individuals. For each dose, the diameter was measured at 5-8 min after injection. Wheal area was calculated as πr^2, where $r = (d_1 + d_2)/4$. The wheal area was corrected for any buffer contribution by subtracting the lesion area of control injections. From a plot of area vs. log peptide dose, the peptide dose at half-maximal area was determined.

EM. At 5 min after injection of the C2b 199-223 peptide, a punch biopsy was taken. The skin fragment was immediately fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3, for 3 h at room temperature. The tissue was then washed overnight in 0.15 M cacodylate buffer, pH 7.3. The tissue was then post-fixed in 1.3% OsO_4 in s-Collodine, pH 7.2. Staining was carried out en bloc in 1.5% uranyl acetate in 0.05 M maleate buffer, pH 6.2. After dehydration in graded ethanol, the tissue was embedded in Epon. Thin sections were cut on an LKB ultratome IV, (LKB Instruments, Inc., Gaithersburg, MD), picked up on carbon-coated grids, lightly stained with lead citrate, and examined in an electron microscope (model 300; Phillips Electronic Instruments, Inc., Mahwah, NJ).

Results

C2 Purification and Digestion with C1s and Plasmin. On SDS-PAGE analysis, the affinity-purified C2 appeared as one band (Fig. 1, lane *1*) with an M_r of 93,000. On SDS-PAGE, (19), C2 and factor B comigrated, independent of the percent acrylamide polymerization. However, alteration of the acrylamide/(bis-acrylamide) ratio

FIGURE 1.   SDS-PAGE of C2 and C2 digests. C2 was digested with C1s (C2/C1s, 500:1 [wt/wt]), or C1s + plasmin (C2/plasmin, 20:1 [wt/wt]) at 37°C for 60 min and analyzed by SDS-PAGE. Lane *1*, C2; lane *2*, C2 + C1s; lane *3*, C2 + C1s plasmin.

from 30:0.8 to 30:0.16 allowed resolution of C2 from factor B. From SDS-PAGE analysis with the lower bis-acrylamide concentration, it was observed that factor B copurified with C2 throughout the purification procedure until the last step. At the last step of purification, factor B was not bound to the C4b column, and was easily separated from C2.

C2 was completely cleaved by C1s into C2a and C2b, which migrated at 70,000 and 32,000 M_r, respectively (Fig. 1, lane 2). The conversion to C2a and C2b was catalyzed by C1s at ratios as high as 5,000 (wt/wt) C2 per C1s as previously reported (20). When C2 was digested with C1s and plasmin, and analyzed by SDS-PAGE, the relative mobility of C2a was the same as that seen after digestion of C1s alone while the mobility of the C2b band was faster (Fig. 1, lane 3). The plasmin digestion product of C2b, C2b', had a relative migration rate corresponding to 28,000 M_r, a molecular mass of 1.7 kD less than that of C2b. The addition of C4 did not change these results. The pattern on SDS-PAGE of C2 digested with C1s and plasmin was the same whether the digestion buffer was PBS (20 mM KH_2PO_4), pH 7.4; veronal buffered saline, pH 7.35, with 5 mM Ca^{2+} and 0.15 mM Mg^{2+}; or 100 mM $NaHCO_3$, pH 8.0; consequently, the proteolytic cleavages of C2 by C1s and plasmin did not require divalent cations. When C2 was incubated with plasmin alone, ~50% of the C2 was converted to C2a and C2b', and no C2b was present (data not shown). The observed conversion of C2b to C2b', therefore, required an enzyme other than C1s, but required neither cations nor the presence of C4.

Analysis of the C2 Digestion Products. When C2 that had been digested with C1s and plasmin was analyzed on a C8 reverse-phase HPLC column, a unique set of two to four small early peaks was observed. (Fig. 2, *A–C*). These closely spaced peaks eluted within a 5% portion of the gradient. When the peptides were analyzed on the more hydrophobic C18 column, the individual peaks eluted again as an unresolved triplet within a restricted portion of the gradient (10%), suggesting the possibility that the peptides were of similar amino acid composition and size. When the HPLC fractions were assayed for biological activity on an isolated rat uterus, all fractions containing these peptides induced contraction (Fig. 3).

NH_2-terminal sequence analysis of C2b' was performed to determine where the C2b was cleaved by plasmin. Comparison of the C2b' NH_2-terminal sequence with that of C2 and the sequence of C2b derived from cDNA (Fig. 4) revealed substantial agreement. Notably, the triplet of amino acids at sequence positions 6–8 of Gln,Asn,Val in the C2b' sequence occurs at positions 6–8 in the NH_2-terminal sequence of C2 and at no other place in the sequence of C2. The difference in molecular weight between the two fragments, C2b and C2b', is then due to a loss of mass from the COOH-terminal portion of C2b.

From the cDNA-derived sequence (26), it can be seen that two lysines and one arginine, which might serve as potential plasmin cleavage sites, are present in this region (Fig. 5 *A*). An incomplete plasmin digest could then result in a mixture of long peptides of 31, 33, and 38 amino acids in length (C2b 186–216, C2b 186–218, and C2b 186–223) and short peptides of two, five, and seven amino acids (C2b 217–118, C2b 219–223, and C2b 217–223). The short peptides were not seen in the HPLC profile as the absorbance of these peptides is too small to be observed with the mass of C2 that was used. It is likely that the early eluting triplet of peptides is the C2b COOH-terminal peptides of 31, 33, and 38 amino acids in length.
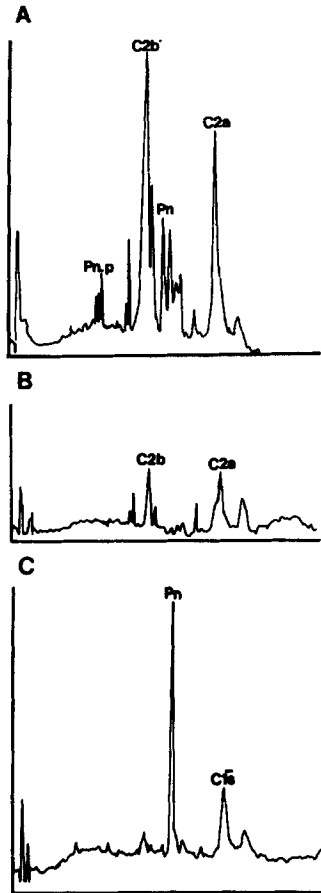
FIGURE 2. Separation of C2 proteolytic fragments on HPLC. Profiles are from HPLC separation of C2 digests on a reverse-phase C8 column, and are plots of absorbance at 215 nm vs. time. For digestion and chromatographic conditions, see text. Pn, plasmin; Pn.P, plasmin-cleaved peptides. (A) C2 + C1s + plasmin (C2/enzyme as in Fig. 1.). C2, 5 nmol. (B) C2 + C1s. C2, 0.1 nmol. (C) C1s + plasmin (plasmin/C1s, 5:1). Plasmin, 1 nmol.
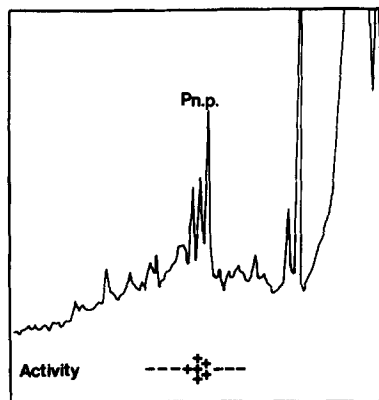

FIGURE 3. Analysis of the C2b peptide fractions from HPLC by the contraction of estrous rat uterus. Peptide-containing fractions from HPLC were pooled, lyophilized, and redissolved in 50 mM acetic acid. For each of the three peptide pools, one half of the total sample was tested for the ability to contract estrous rat uterus. Each sample of peptide induced contraction. The extent of contraction for each peptide, relative to the other two peptide samples, is shown as denoted by (+) least, (+ +) intermediate, and (+ + +) most.

AA position: 1   2   3   4   5   6   7   8   9   10 11
C2b' sequence: ALA PRO --- --- PRO³ GLN ASN VAL --- ILE ---
C2 sequence, protein[1]:   ALA PRO SER --- PRO GLU ASN VAL --- LEU
C2 sequence, cDNA:[2]   ALA PRO SER CYS PRO GLN ASN VAL ASN ILE SER

FIGURE 4. NH₂-terminal sequence analysis of C2b' and a comparison of this sequence to published sequences of the NH₂ terminus of C2. (---) No amino acid detected at that cycle in the sequence analysis. (1) Taken from Kerr and Porter (34), 1979. (2) Taken from Bentley (26), 1986. (3) A small amount of Cys was also present in this cycle.

Synthesis and Activity of C2 Peptides. Peptides were synthesized to test in biological assays. The putative COOH-terminal peptides of 31, 33, and 38 amino acids in length have common NH₂ termini, but distinct COOH termini. Peptides with COOH termini corresponding to the COOH terminus of C2b plasmin cleavage products and of increasing amino acid length were synthesized (Fig. 5 B) and tested by intradermal injections on human skin. For a given peptide dose, the extent of edema surrounding the injection site was directly proportional to the length of the peptide injected (Table II). The smaller peptides of <10 amino acids required doses of 65 nmol or more to achieve a half-maximal response, whereas 20 nmol or less of the peptides C2b 207–223 (17 amino acids) and C2b 199–223 (25 amino acids) were required to achieve the same response. For the 25–amino acid peptide, a half-maximal response was observed with 1.5 nmol of peptide.

When synthetic peptides were tested for the ability to contract estrous rat uterus, a different correlation of activity to peptide length was found. The penta- and heptapeptides from the COOH terminus of C2b induced the contraction of estrous rat uterus, while the peptide 212–216 had no activity at the concentrations tested (≤650 nmol). No substantial differences in activity were observed for peptides of 5–19 amino acids in length. There was insignificant change in the response induced on the isolated rat uterus by the heptapeptide as compared with the pentapeptide. The pentapeptide, C2b 219–223, induced contraction of the rat uterus in amounts of 40–220 nmol and greater with a half-maximal contraction occuring at 125 nmol. The longer

A

C2b C-terminus:

-NH-Arg^V GlnProTyrSerTyrAspPheProGluAspValAlaProAlaLeuGlyThrSerPheSer
         185   190   195   200   205

HisMetLeuGlyAlaThrAsnProThrGlnLys^V ThrLys^V GluSerLeuGlyArg-CO₂H
         210   215   220   223

B
1   C2b 212-216
2   C2b 219-223
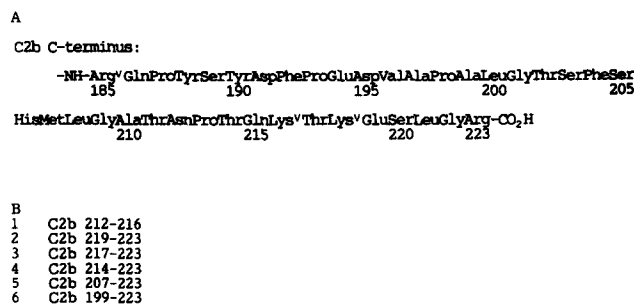3   C2b 217-223
4   C2b 214-223
5   C2b 207-223
6   C2b 199-223

FIGURE 5. Possible plasmin cleavage sites in C2b and the sequence of the proposed active site peptides. (A) Possible plasmin cleavage sites in the C2b COOH-terminal sequence, and the putative sequence of the HPLC-fractionated peptides. (V) Putative plasmin-cleavage site. The amino acid residue numbers are assigned from the cDNA sequence of Bentley (26). (B) Synthetic peptides that correspond to putative COOH termini of C2 vasoactive peptides.

TABLE II

Effect of Peptide Length on the Enhancement of Human Skin Vasopermeability

Peptides	Dose at half-maximal response
	nmol
C2b 219–223	≫250
C2b 214–223	65
C2b 207–223	17.5
C2b 199–223	1.5

peptide of C2b 217–223 induced the contraction of the rat uterus at doses of 100–600 nmol and greater with the half-maximal contraction occuring at 240 nmol. The C2b 199–223 peptide of 25 amino acids in length was more active; it induced a like contraction of the estrous rat uterus at a concentration 1:20 that of the shorter peptides.

On permeability-enhancing tests with guinea pig skin, a similar dependence on peptide length as that observed on the rat uterus was found. The small peptides of five and seven amino acids in length gave half-maximal lesion areas at doses of 340 and 330 nmol, respectively, and in both cases the range of effective doses was 100–1,000 nmol. Except for the C2b 199–223 peptide of 25 amino acids, the longer peptides showed no increase in specific activity; the C2b 199–223 peptide was ~100 times more active than shorter peptides.

The permeability-enhancing effects of peptide C2b 199–223 on human and guinea pig skin was tested in the presence of antihistamines. Tripelennamine hydrochloride (150 mg), was administered before human skin testing, and 1.5 h later, serial dilutions of C2b 199–223 were injected intradermally. At concentrations of peptide required for a submaximal response, the area of the resultant wheal was not affected by the drug (Table III). When guinea pigs were administered the antihistaminic drug, polarimine (1 mg/kg), before testing, the resultant bluing was less intense and polarimine appeared to enhance the vasopermeability induced by this peptide (Table IV).

In electron micrographs of the lesion induced by injection of the peptide C2b

TABLE III

The Effect of Triplennamine Hydrochloride (PBZ) on the Enhancement of Human Skin Vascular Permeability by C2b 199–223

Dose	– PBZ	+ PBZ
	mm²	*mm²*
C2b 199–223:		
13 nmol	99	57
6.5	104	71
3.8	78	78
1.9	38	33
1.0	44	16
Histamine:		
1 µg	99	24

TABLE IV

The Effect of Polarimine on the Enhancement of Guinea Pig Skin
Vasopermeability by C2b 199–223

Dose	– Polarimine	+ Polarimine
	mm^2	mm^2
C2b 199–223:		
125 nmol	75	83
62	31	115
31	31	115
15.6	25	47
7.8	19	35
3.9	25	21
2.0	–	–
Histamine:		
6 µg	323	16

199–223, evidence of enhanced vascular permeability was present (Fig. 6). Gaps at endothelial cell junctions were present and the endothelial cell nuclei were distorted so as to suggest that they were contracted.

Discussion

When C2 is digested with C1s and plasmin, small peptides are released from C2b that induced contraction of estrous rat uterus. Since the NH_2-terminal sequence of plasmin-digested C2b' was identical with the NH_2-terminal sequence of C2, it appears that the plasmin-cleaved peptides were derived from the COOH-terminus of C2b (Fig. 7). Synthetic peptides identical in sequence to the proposed active site of C2b were synthesized and shown to induce contraction of estrous rat uterus and to enhance both human and guinea pig skin vascular permeability. The C2b peptide, C2b 199–223, of 25 amino acids in length substantially enhanced vascular permeability in human skin at 1 nmol.

The ability of the synthetic peptides to enhance the vascular permeability of both human and guinea pig skin was found to proceed in the presence of antihistiminic drugs, which distinguishes them from the complement anaphylatoxins, C3a, C4a, and C5a (27–31).

When isolated C2 was digested, the cleavage of the peptides from C2b by plasmin did not require divalent cations or C4. Whether or not C4 enhances the kinetics of cleavage was not determined in the 60-min incubations used in this study.

A vasoactive fragment analogous to the peptide from C2b is not present in factor B, thus confining this particular vasoactivity to the classical complement pathway. Factor B, which functions in the alternative pathway C3 convertase in a matter analogous to the function of C2 in the classical pathway C3 convertase, has been shown to have 40% amino acid sequence homology with C2, as well as a similar domain structure (26). Activation of factor B with C3b and factor D yields two fragments that display relative mobilities on SDS-PAGE of 68,000 M_r (Bb) and 28,000 M_r (Ba). Addition of plasmin, however, does not change the mobilities of either fragment (data not shown).

FIGURE 6.   Electron micrograph of a skin lesion dermal micro vessel after injection of C2b 207–223. Dermal micro vessel ($\times$ 10,000) with an RBC in its lumen. Gaps between the two endothelial cells (*arrow heads*) are observed. The irregular contour of the endothelial cell nucleus (*N*) suggests that endothelial cell contraction has occurred.

When C2b synthetic peptides were tested in the guinea pig skin and rat uterus assays, only a peptide of 25 amino acids in length was more active than peptides of 5–19 amino acids in length. This contrasts with the effects of the peptides of increasing length on human skin in which a steady increase in activity with increasing
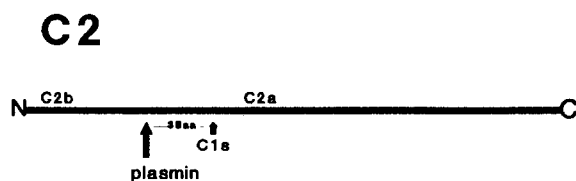
C2

FIGURE 7.   C2 and its fragments that are digestion products of serine proteinase cleavage.

peptide length was observed. The difference may be due to key differences between the C2 sequences of rat, guinea pig, and human in this region. As C2 sequence of this region from these animals is not available, we can not make a direct comparison. The synthetic C2 peptides have been shown to have multiple activities. A summary of the dose responses for the peptides of varying amino acid length is shown in Table V.

With the exception of the COOH-terminal arginine residue, the sequence of the putative plasmin-derived 38–amino acid active peptide of C2b demonstrates no sequence homology to bradykinin. There is also no sequence homology with C4a and C3a, and only minimal sequence homology with C5a. The three amino acids of the COOH terminus of C5a and C2b, Leu,Gly,Arg, are identical. Both peptides require these COOH-terminal residues for activity.

The plasmin-derived peptide from C2b that we describe here shares many features with that of the previously described peptide kinin-permeability factor isolated from HANE plasma (2). In the 38–amino acid residues are multiple sites for either chymotryptic (three sites at C2b residues 189, 191, and 205) and tryptic cleavage (two sites at C2b residues 216 and 218). The activity of the HANE peptide was found to be sensitive to both these serine esterases. This contrasts with the susceptibility of bradykinin only to chymotrypsin. At pH 8.6, the 38-residue peptide would have a net charge of zero in contrast to bradykinin which carries a net charge of +2. Thus, bradykinin would be more electropositive on electrophoresis than this peptide, correlating another reported difference between bradykinin and the peptide from HANE plasma.

The results of the human skin test coupled with the above correlatives suggest that a C2b vasoactive fragment may likely play a role in the pathogenesis of edema associated with C1 inhibitor deficiency. In HANE, assay of plasma complement components during and after attack revealed normal levels of C3–9 (1, 32), suggesting that the swelling episodes are not mediated by C3a or C5a. Furthermore, symptoms can not be alleviated by antihistaminic drugs, again suggesting the lack of C3a and C5a involvement. The dose of a synthetic C2b peptide of 25 amino acids that causes substantial edema is equivalent to the amount of precursor C2 in ~8 ml of collected blood, which represents ~0.2% the total blood volume in a single individual. These results are then in keeping qualitatively with previous experiments in which C2-deficient guinea pigs were supplemented with functionally pure C2, and a C2-dependent vasopermeability response was observed with as little added C2 as 10%

TABLE V

Relative Biological Activities of C2 Peptides

	Assay		
Peptide	Rat uterus contraction	Guinea pig vasopermeability	Human skin vasopermeability
	μM	μmol	μmol
C2b 219–223 or 217–223	10	0.5	0.5
C2b 214–223	10	0.5	0.1
C2b 207–223	10	0.5	0.015
C2b 119–223	1.0	0.005	0.001

of that found in a normal guinea pig (5). Similarly, when HANE plasma was enriched with C2, enhanced kinin generation occurred (33). Therefore, there is adequate intact C2 to generate the active C2b peptide in vivo, and these data suggest that it is possible to generate a similar dose response to the above skin test by cleavage of C2 in vivo. Consequently, the role of C2 may be important in the pathogenesis of angioedema.

We find that a second serine proteinase is required for the release of the C2 vasoactive fragment as previously reported (33). The amino acid length of the active physiological C2 fragment is not yet known, nor is it known what might be the in vivo enzyme that releases the vasoactive peptide from its C2 precursor. Although plasmin is inhibited by C1 inhibitor, it is also complexed with α_2-macroglobulin and α_2-antiplasmin. Two aspects of plasmin metabolism still make it a candidate for the in vivo enzyme. The swelling episodes in angioedema are often localized within a small area of the body, and are typically induced by minor trauma. The local boundaries of the lesions may be a consequence of the preferential regulation of plasmin activity in the extravascular spaces by C1 inhibitor. Alternatively, a tissue activation mechanism, such as that of a tissue plasminogen activation, might provide only local concentrations of active enzyme.

Summary

Synthetic peptides that correspond to the COOH-terminal portion of C2b enhance vascular permeability in human and guinea pig skin. In human studies, 1 nmol of the most active peptide of 25–amino acid residues produced substantial local edema. A pentapeptide and a heptapeptide corresponding to the COOH-terminal sequence of C2b each induced contraction of estrous rat uterus in the micromole range; a peptide of 25 amino acids from this region induced a like contraction of rat uterus at a concentration 20-fold lower than the smaller peptides. The vascular permeability of guinea pig skin was enhanced by doses of these synthetic peptides in a similar fashion as that observed for the concentration of rat uterus.

The induction of localized edema by intradermal injection in both the guinea pig and the human proceeds in the presence of antihistaminic drugs, suggesting that there is a histamine-independent component to the observed increase in vascular permeability.

Cleavage of C2 with the enzymic subcomponent of C1, C1s, yields only C2a and C2b, and no small peptides, whereas cleavage of C2 with C1s and plasmin yields a set of small peptides. These plasmin-cleaved peptides are derived from the COOH terminus of C2b, and they induce the contraction of estrous rat uterus.

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